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CORRELATION AND BODY PROPORTIONS IN MATURE MICE OF THE GENUS PEROMYSCUS*

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Received September 27, 1940

IN THE present investigation, three species and several subspecies of mice belonging to the genus Peromyscus are compared with respect to the amounts of simple correlation and the size of the ratios between certain body and skeletal measurements. The means, standard deviations, and coefficients of variation are also given for all but one of the measurements. The only exception is ear length, which was added later and appears only in the tables of means and of ratios.

These data, in so far as they have a bearing on the subject, are presented as evidence regarding the relative importance of general, group, and special size factors. Evidence for the existence of these three types of size factors has been convincingly presented by WRIGHT (1932).

SUMNER (1932) and DICE (1932, 1933, 1938, 1939a, 1939b) have made extensive studies on variation in mice of the genus Peromyscus and have demonstrated that subspecific characters are inherited, but that there is considerable variation in mice of the same subspecies from different localities.

MATERIALS AND METHODS

Forty-two stocks of mice representing three species and ten subspecies were employed in these statistical analyses. The widespread deer mouse, *Peromyscus maniculatus*, is represented by one or more stocks in each of eight subspecies—namely, *artemisiae*, *bairdii*, *blandus*, *gambelii*, *osgoodi*, *rubidus*, *rufinus*, and *sonoriensis*. The wood mouse, *Peromyscus leucopus*, is presented by eight stocks of the one subspecies, *noveboracensis*. The cactus mouse, *Peromyscus eremicus*, is illustrated by the subspecies *eremicus* from four localities. The classification and range of these mice have been described by OSGOOD (1909).

The funds expended in collecting these mouse stocks were furnished in most part by the CARNEGIE INSTITUTION OF WASHINGTON, the UNIVERSITY OF MICHIGAN Museum of Zoology, and the WILLIAM P. HARRIS, JR., RESEARCH FUND. The stocks were collected by: LEE R. DICE, RUTH and ARTHUR SVIHLA, PAUL A. MOODY, H. J. LERAAS, HOBART M. SMITH, PHILIP F. ALLAN, PAUL HICKIE, G. W. BRADT, F. B. SUMNER, ADOLPH STEBLER, ADOLPH MURIE, O. J. MURIE, B. T. OSTENSON, R. R. HUESTIS, A. J. NICHOLSON, W. L. STRUNK, L. C. STEGEMAN, A. H. HOWELL, and PHILIP BLOSSOM.

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The expense of rearing the animals was defrayed by the UNIVERSITY OF MICHIGAN through the Museum of Zoology, Faculty Research Fund, and the Laboratory of Vertebrate Genetics. Additional aid was given by grants from the National Research Council and by assistants furnished by the Federal National Youth Administration and Works Progress Administration.

The methods used in obtaining the body and skull measurements of these mice have been described in detail by DICE (1932). To avoid possible misunderstanding, a brief description of each measurement will be given here. Tail length is the distance from the upper base of the tail to the tip of the tail, not including the hairs; body length is derived by subtracting tail length from the total length, total length being the distance from the tip of the nose to the end of the tail, not including the tail hairs; ear length is the length of the left ear from the notch at the lower end of the ear opening to the tip of the ear; length of femur is the greatest length of the left femur from the condyles to the end of the great trochanter; foot length is length of the left hind foot from the heel to the end of the middle claw; skull length is the distance from the left occipital condyle to the most anterior part of the left premaxilla. The point taken on the premaxilla is slightly below the nasal and is not in the median line of the skull; condyle-zygoma is the distance from the left condyle to the angle at the most anterior part of the zygomatic process of the left maxilla; mandibular length is the greatest length of the left mandible, from condyle to end of incisor; skull width is the width of the skull across the mastoid processes.

The measurements used in this study were all taken from mice of the one year class (36 to 78 weeks of age when killed). There is not enough difference in size between males and females to justify separate treatment of the sexes.

Simple correlations and ratios were calculated for the following pairs of measurements: tail length/body length, skull length/body length, femur length/foot length, mandibular length/skull length, condyle-zygoma/skull length, and skull width/skull length. Ratios alone were calculated for ear length/skull length. The average correlations and ratios for the several subspecies and species are given and these average values will be used more than the individual values in the discussion. The number of individual measurements available for each of the stocks ranged from 44 to 164 and in most stocks was about 70.

It should be emphasized that the mice used in this study were all bred in the laboratory from unselected parents. These laboratory-bred mice were raised under uniform environmental conditions and were all of a nearly similar age when killed. It is believed, therefore, that their measurements and correlations are fully comparable.

The means and standard deviations are not discussed in detail. Values given for these stocks in the publications of DICE may differ slightly from those given in the present paper due to the use of different numbers of mice.

CORRELATIONS BETWEEN MEASUREMENTS

The correlations between certain measurements of Peromyscus are given in table 1. The average correlations for the various subsepecies and species in table 2 were obtained by transforming the simple correlations in table 1 into z values and securing the weighted averages. In both tables the values of n are included. In table 1, a single + sign after a coefficient means that it equals or is beyond the 5 percent level of significance, while two plus signs (++) indicate that it equals or is beyond the 1 percent level of significance. A coefficient with no + sign after it is not statistically significant. All average coefficients in table 2 are significant statistically, so no + signs are used.

After the correlations had been completed, the author's attention was called to the fact that the correlations between some of the pairs of measurements had little significance because one of the variables is a part of the other variable. For example, in the skull length and body length correlations, skull length is a part of body length, for body equals trunk plus head. The same criticism held true for the mandible and skull length correlations as well as for those of condyle-zygoma with skull length. The true correlations in these three sets were obtained by using the following formulae: $\sigma B^2 = \sigma C^2 + \sigma A^2 - 2\sigma A \sigma C r_{AC}$; $\sigma C^2 = \sigma A^2 + \sigma B^2 + 2\sigma A \sigma B r_{AB}$. For example, in the skull length and body length correlations C is a whole (body), A is the skull, and B is the trunk. By means of the first formula, σB^2 and σB are secured. The true value of the correlation is obtained by substituting in the second formula, the equation being solved for r_{AB}.

Slight errors introduced by the fact that the mandible measurement is not taken exactly along the longitudinal axis like the skull length measurement would obviously be less serious than those that would result from using the original spurious correlations.

These three sets of correlations thus become skull length and body length minus skull length; mandible length and skull length minus mandible length; condyle-zygoma and skull length minus condyle-zygoma length. In tables 1 and 2, however, the original headings are used because of their brevity; the reader should bear this fact in mind.

The correlations between the same pairs of measurements in the various stocks differ considerably in magnitude. For example, the simple correlations between tail length and body length vary in the species *maniculatus* from −.16 in *P. m. sonoriensis* from Winslow, Arizona, to +.66 in *P. m.*

TABLE 1

Correlations between certain measurements of Peromyscus.

SPECIES	SUBSPECIES	LOCALITY	TAIL / BODY		SKULL LENGTH / BODY		FEMUR / FOOT		MANDIBLE / SKULL LENGTH		CONDYLE-ZYGOMA / SKULL LENGTH		SKULL WIDTH / SKULL LENGTH	
			n	r	n	r	n	r	n	r	n	r	n	r
maniculatus	*artemisiae*	Godman Springs, Wash.	66	+.45++	67	+.50++	60	+.36++	63	+.71++	63	+.10	66	+.11
"	"	Palouse Falls, Wash.	86	+.28++	76	+.53++	85	+.47++	83	+.71++	84	+.24+	83	+.50++
"	"	Troy, Idaho	74	+.09	54	+.28+	63	+.40++	57	+.49++	58	+.28+	59	+.71++
"	*bairdii*	Ann Arbor, Mich.	106	+.66++	82	+.55++	94	+.59++	80	+.78++	83	+.26+	80	+.55++
"	"	Alexander, Iowa	97	+.51++	63	+.66++	74	+.41++	62	+.83++	63	+.31+	58	+.75++
"	"	Barrett, Minn.	88	+.60++	81	+.46++	85	+.08	78	+.75++	80	+.29++	77	+.55++
"	"	Onaga, Kansas	85	+.37++	79	+.47++	80	+.11	80	+.71++	80	+.26+	80	+.66++
"	"	Sand Point, Mich.	82	+.03	77	+.38++	78	+.32++	73	+.62++	77	+.06	73	+.04
"	"	Lake Village, Ind.	78	+.45++	70	+.51++	74	+.34++	70	+.71++	70	+.22	69	+.71++
"	"	Alamogordo, N. Mex.	107	+.28++	88	+.64++	86	+.57++	85	+.73++	88	+.55++	85	+.65++
"	*blandus*	White Sands, N. Mex.	99	+.10	59	+.55++	61	+.53++	57	+.60++	59	+.05	58	+.52++
"	*gambelii*	La Jolla, Calif.	164	+.09	149	+.47++	159	+.34++	148	+.72++	149	+.14	149	+.48++
"	*osgoodi*	Cottonwood Basin, S. Dak.	49	+.19	40	+.62++	52	+.30++	52	+.87++	52	+.49++	52	+.64++
"	"	Mandan, N. Dak.	96	+.05	93	+.50++	95	+.39++	95	+.77++	95	+.24+	95	+.70++
"	"	Otis, S. Dak.	95	+.22+	96	+.51++	94	+.24+	96	+.74++	96	+.58++	96	+.40++
"	"	Jackson, Wyo.	114	+.30++	70	+.52++	68	+.62++	70	+.80++	70	+.41++	69	+.63++
"	"	Logan Canyon, Utah	63	+.34++	63	+.13	61	+.51++	64	+.52++	63	+.53++	63	+.71++
"	"	Taylor, N. Dak.	82	+.36++	81	+.59++	80	+.32++	81	+.79++	81	+.15	18	+.56++
"	"	Gregory, S. Dak.	94	+.10	89	+.53++	92	+.40++	92	+.78++	93	+.38++	93	+.61++
"	*rubidus*	Eugene, Oregon	73	+.59++	92	+.59++	93	+.53++	92	+.78++	97	+.31++	92	+.62++
"	*rufinus*	La Veta, Colo.	78	+.38++	59	+.68++	70	+.35++	60	+.81++	60	+.41++	59	+.67++
"	"	Deadman Flat, Ariz.	75	+.66++	84	+.68++	82	+.31++	86	+.87++	78	+.53++	79	+.62++
"	"	Little Spring, Ariz.	115	+.34++	109	+.64++	108	+.26++	104	+.69++	103	+.38++	97	+.58++
"	"	Ice Caves, Ariz.	57	+.39++	64	+.59++	55	+.33+	59	+.85++	66	+.29+	58	+.65++
"	"	Bonito Canyon, N. Mex.	65	+.34++	52	+.22	54	+.36++	70	+.75++	63	+.37++	54	+.63++
"	"	Cloudcroft, N. Mex.	110	+.56++	83	+.62++	105	+.32++	94	+.71++	101	+.50++	91	+.49++
"	*sonoriensis*	Miller Canyon, Ariz.	74	+.56++	73	+.68++	67	+.40++	77	+.75++	74	+.43++	71	+.68++
"	"	Winslow, Ariz.	59	−.16	57	+.50++	56	+.41++	57	+.72++	56	+.21	54	+.54++
"	"	San Felipe, Calif.	78	+.31++	78	+.60++	90	+.31++	88	+.81++	90	+.56++	83	+.56++
"	"	Victorville, Calif.	61	+.18	60	+.35++	59	+.13	57	+.87++	60	+.26+	57	+.43++
leucopus	*noveboracensis*	Ann Arbor (W), Mich.	99	+.33++	94	+.53++	95	+.27++	94	+.79++	95	+.14	93	+.53++
"	"	Ann Arbor (St), Mich.	47	+.08	51	+.48++	48	+.04++	48	+.78++	44	+.18	53	+.43++
"	"	Ann Arbor (D), Mich.	56	+.26	52	+.38++	52	+.51++	50	+.91++	53	+.20	51	+.64++
"	"	Cedarvale, N. Y.	85	+.40++	83	+.61++	83	+.38++	84	+.75++	84	+.18	84	+.58++
"	"	Decker, Ind.	71	+.40++	80	+.39++	84	+.61++	66	+.88++	71	+.41++	75	+.53++
"	"	Greenland, N. H.	85	+.38++	69	+.48++	81	+.19	71	+.63++	72	+.54++	68	+.60++
"	"	Canebrake, Ind.	62	+.42++	69	+.62++	68	+.34++	68	+.80++	74	+.19	68	+.48++
"	"	Asheville, N. C.	66	+.37++	59	+.40++	61	+.49++	59	+.72++	60	−.17	62	+.50++
eremicus	*eremicus*	Tumamoc Hill, Ariz.	98	+.28++	122	+.29++	124	+.52++	119	+.62++	122	+.40++	120	+.45++
"	"	Tortollito Mts., Ariz.	105	+.24+	93	+.45++	96	+.36++	94	+.72++	95	+.22+	94	+.61++
"	"	Pima Canyon, Ariz.	84	+.19	83	+.61++	91	+.57++	84	+.73++	83	+.35++	84	+.57++
"	"	1000-Palm Canyon, Calif.	73	+.15	99	+.57++	99	+.54++	100	+.73++	100	+.37++	99	+.61++

+ 5% level of significance. ++ 1% level of significance.

bairdii from Ann Arbor, Michigan. The remainder of these correlations fall between these extremes, and although they are small, most of them are statistically significant. By referring to the average correlations in table 2, we find that within the species *maniculatus*, the average coefficient varies from +.09 in the subspecies *P. m. gambelii* to +.59 in the subspecies *P. m. rubidus*. Since each of the subspecies is represented by only one stock, the coefficients in this case are not averages but are included in the table for comparison. In subspecies which are represented by three or more stocks, the correlations range from +.26 to +.47 in the species *P. maniculatus*. In the three species *P. maniculatus*, *P. leucopus*, and *P. eremicus* the average correlations are +.34, +.35, and +.22, respectively, the grand average being +.33. Since body length is a good measure of general body size, the small magnitude of these correlations indicates that tail length is determined largely by factors other than those that determine general body size—in other words, by special size factors.

The correlations between skull length and body length (minus skull length) in tables 1 and 2 represent the true correlations. The correlations are somewhat larger than those found between tail and body. In table 1, these correlation coefficients vary from +.13 in *P. m. osgoodi* from Logan Canyon, Utah, to +.68 in two stocks of *P. m. rufinus* and one stock of *P. m. sonoriensis*. In table 2, the average coefficients of the subspecies fluctuate from +.46 in *P. m. artemisiae* to +.61 in *P. m. blandus* and *P. m. rufinus*. The average species correlations for the three species *P. maniculatus*, *P. leucopus*, and *P. eremicus* are +.54, +50 and +47, respectively, the grand average being +.52. These data indicate that only approximately 27 percent (r^2) of the variation in skull length and body (trunk) length is due to common genetic or environmental causes.

The correlations between femur length and foot length are, in general, small but statistically significant. Referring to table 1, we find that the coefficients for the individual stocks range from +.08 in *P. m. bairdii* from Barrett, Minnesota, to +.64 in *P. l. noveborascensis* from Ann Arbor (St), Michigan. The species averages for *P. maniculatus*, *P. leucopus*, and *P. eremicus* are +.37, +.42, and +.50, the grand average being +.40 (table 2). These results show that foot length, for the most part, varies independently of femur length.

The correlations between mandible length and skull length (minus mandible length) proved to be the largest of any of those studied and ranged from +.49 in *P. m. artemisiae* from Troy, Idaho, to +.91 in *P. l. noveborascensis* from Ann Arbor (D), Michigan (table 1). The average correlations of the ten subspecies (table 2) vary from +.66 to +.79. The species's averages are +.75, +.79, and +.70, respectively, for the three species *P. maniculatus*, *P. leucopus*, and *P. eremicus*, the grand average being +.75.

TABLE 2

Average correlations between certain measurements of several species and subspecies of Peromyscus.

SPECIES	SUBSPECIES	NO. OF STOCKS AVERAGED	TAIL / BODY		SKULL LENGTH / BODY		FEMUR / FOOT		MANDIBLE* / SKULL LENGTH		CONDYLE-ZYGOMA / SKULL LENGTH		SKULL WIDTH / SKULL LENGTH	
			n	r	n	r	n	r	n	r	n	r	n	r
maniculatus	*artemisiae*	3	226	+.27	197	+.46	123	+.42	203	+.66	205	+.21	208	+.47
"	*bardii*	6	536	+.47	452	+.50	485	+.43	443	+.74	453	+.23	437	+.57
"	*blandus*	2	206	+.20	147	+.61	147	+.55	142	+.68	147	+.37	143	+.60
"	*gambelii*	1	164	+.09	149	+.47	159	+.34	148	+.72	149	+.14	149	+.48
"	*osgoodi*	7	593	+.22	541	+.50	542	+.40	550	+.76	550	+.40	549	+.61
"	*rubidus*	1	73	+.59	92	+.59	93	+.53	92	+.78	97	+.31	92	+.62
"	*rufinus*	6	500	+.46	451	+.61	474	+.31	473	+.78	371	+.42	438	+.60
"	*sonoriensis*	4	272	+.26	268	+.56	272	+.32	279	+.79	280	+.40	265	+.57
	Species average			+.34		+.54		+.37		+.75		+.34		+.58
leucopus	*noveboracensis*	8	571	+.35	557	+.50	572	+.42	540	+.79	553	+.22	554	+.54
eremicus	*eremicus*	4	360	+.22	397	+.47	410	+.50	397	+.70	400	+.34	397	+.56
	Genus average			+.33		+.52		+.40		+.75		+.32		+.57

* See text of article for explanation of these correlations.

Therefore, approximately 56 percent (r^2) of the size variations in mandible length and skull length (minus mandible length) may be attributed to general or group size factors. Apparently, special size factors also play an important part in determining the size of these parts.

The true or net correlation coefficients between condyle-zygoma and skull length (minus condyle-zygoma length) are listed in the fifth columns of tables 1 and 2. These coefficients are small, being of about the same magnitude as those for tail with body, and they vary from +.05 in *P. m. blandus* from White Sands, New Mexico, to +.58 in *P. m. osgoodi* from Otis, S. Dakota (table 1). The average coefficients (table 2) for the three species *P. maniculatus*, *P. leucopus*, and *P. eremicus* are +.34, +.22, and +.34, respectively. The grand average is +.32.

The correlations between skull width and skull length are only moderate in size, most of them being statistically significant. The average values (table 2) for the three species *P. maniculatus*, *P. leucopus*, and *P. eremicus* are +.58, +.54, and +.56, respectively; the grand average is +.57. Obviously some of the size factors affecting skull length also have an effect upon skull width. Such factors may be either general or group factors. Only 32 percent of the variation in these two variables is caused by such factors. The remainder may be attributed to special genetic or environmental factors.

RATIOS BETWEEN MEASUREMENTS

Ratios were calculated between some of the body and skeletal measurements for the purpose of comparing the relative proportion between these parts in different stocks. For example, the tail length/body length ratio was computed by dividing mean tail length by mean body length. It is true that the ratio of two means may differ from the mean of individual ratios especially if the coefficients of variability are large. With several stocks, a ratio was obtained in both ways, and since they checked to three or four decimal places, the easier method was used. For calculating the standard errors of the ratios, the following formula was used:

$$\text{S.E. } B/A = \frac{B/A\sqrt{V_A^2 + V_B^2 - 2V_A V_B r_{AB}}}{\sqrt{n}}$$

where B/A = the ratio, V/A = coefficient of variability of A/100, and r_{AB} = correlation coefficient between A and B.

The ratios of the individual stocks are shown in table 3. There are so many significant differences between these ratios, however, that a discussion of them is somewhat confusing. To simplify matters, the ratios of the individual stocks were averaged to obtain the average ratio for each subspecies, each species, and for all the stocks. Since the collections of mice

TABLE 3

Ratios and their standard errors between certain measurements of Peromyscus.

SPECIES	SUBSPECIES	LOCALITY	TAIL / BODY	SKULL LENGTH / BODY	FEMUR / FOOT	EAR / SKULL LENGTH	MANDIBLE / SKULL LENGTH	CONDYLE-ZYGOMA / SKULL LENGTH	SKULL WIDTH / SKULL LENGTH
maniculatus	*artemisiae*	Godman Springs	.8783± .0063	.2683± .0014	.7819± .0051	.7312± .0039	.6446± .0014	.7091± .0027	.4382± .0016
"	"	Palouse Falls	.7346± .0051	.2554± .0010	.8035± .0032	.7204± .0036	.6537± .0013	.7086± .0021	.4378± .0010
"	"	Troy	.8506± .0063	.2609± .0012	.7946± .0036	.7200± .0038	.6513± .0020	.7202± .0024	.4344± .0008
"	*bairdii*	Ann Arbor	.7063± .0035	.2725± .0013	.7878± .0033	.6182± .0033	.6615± .0017	.7316± .0027	.4576± .0014
"	"	Alexander	.7295± .0037	.2768± .0013	.8135± .0042	.6273± .0038	.6743± .0015	.7274± .0034	.4492± .0014
"	"	Barrett	.6345± .0039	.2524± .0012	.8220± .0039	.6418± .0032	.6740± .0012	.7290± .0022	.4530± .0012
"	"	Onaga	.6281± .0042	.2599± .0009	.7998± .0050	.6466± .0024	.6662± .0013	.7295± .0023	.4606± .0011
"	"	Sandpoint	.6127± .0050	.2551± .0009	.8110± .0036	.6599± .0028	.6641± .0014	.7317± .0021	.4583± .0014
"	"	Lake Village	.6540± .0039	.2580± .0010	.7840± .0039	.6251± .0034	.6867± .0015	.7315± .0026	.4486± .0010
"	*blandus*	Alamogordo	.7524± .0064	.2593± .0016	.8051± .0053	.7452± .0040	.6575± .0016	.7145± .0021	.4393± .0011
"	"	White Sands	.7416± .0063	.2582± .0015	.7996± .0054	.7016± .0035	.6628± .0020	.7177± .0030	.4345± .0012
"	*gambelii*	La Jolla	.7941± .0054	.2538± .0008	.8040± .0026	.7553± .0026	.6550± .0008	.7140± .0015	.4420± .0008
"	*osgoodi*	Cottonwood Basin	.7001± .071	.2560± .0012	.7737± .0044	.6617± .0043	.6670± .0013	.7231± .0026	.4447± .0013
"	"	Mandan	.6632± .0053	.2505± .0009	.8109± .0032	.6660± .0033	.6036± .0013	.7210± .0025	.4510± .0009
"	"	Otis	.6707± .0047	.2510± .0010	.7871± .0032	.7137± .0036	.6672± .0012	.7159± .0016	.4400± .0012
"	"	Jackson	.8912± .0062	.2776± .0016	.8047± .0036	.7508± .0040	.6629± .0014	.7101± .0028	.4528± .0013
"	"	Logan Canyon	.7572± .0063	.2580± .0015	.7926± .0037	.7340± .0041	.6555± .0021	.7061± .0021	.4440± .0011
"	"	Taylor	.6270± .0045	.2525± .0009	.8365± .0048	.6576± .0029	.6031± .0012	.7235± .0024	.4460± .0011
"	"	Gregory	.6406± .0052	.2548± .0011	.8290± .0038	.6403± .0044	.6716± .0015	.7203± .0024	.4512± .0013
"	*rubidus*	Eugene	.9606± .0068	.2607± .0011	.7612± .0030	.7185± .0039	.6485± .0013	.7161± .0026	.4386± .0011
"	*rufinus*	La Veta	.8391± .0068	.2725± .0013	.7831± .0039	.7450± .0051	.6603± .0018	.7140± .0025	.4441± .0011
"	"	Deadman Flat	.6523± .0050	.2580± .0011	.8013± .0042	.7849± .0043	.6552± .0012	.7126± .0024	.4445± .0013
"	"	Little Spring	.6521± .0037	.2564± .0008	.7955± .0035	.7509± .0032	.6556± .0012	.7153± .0022	.4445± .0009
"	"	Ice Caves	.6751± .0058	.2529± .0009	.8147± .0052	.7704± .0045	.6550± .0015	.7148± .0027	.4454± .0011
"	"	Bonito Canyon	.7176± .0058	.2628± .0017	.7890± .0047	.7424± .0046	.6567± .0014	.7122± .0027	.4430± .0013
"	"	Cloudcroft	.8133± .0047	.2803± .0012	.7776± .0037	.7553± .0038	.6609± .0015	.7084± .0019	.4347± .0012
"	*sonoriensis*	Miller Canyon	.6398± .0038	.2523± .0015	.8128± .0039	.6958± .0040	.6541± .0016	.7242± .0025	.4428± .0012
"	"	Winslow	.6542± .0051	.2567± .0009	.7998± .0042	.7436± .0056	.6554± .0018	.7169± .0028	.4456± .0015
"	"	San Felipe	.7831± .0067	.2521± .0011	.7959± .0037	.7432± .0040	.6461± .0013	.7225± .0021	.4138± .0012
"	"	Victorville	.8263± .0061	.2826± .0013	.8125± .0047	.6661± .0035	.6477± .0013	.7249± .0030	.4504± .0016
leucopus	*noveboracensis*	Ann Arbor (W)	.8441± .0051	.2585± .0012	.8146± .0034	.6810± .0027	.6608± .0011	.7146± .0023	.4360± .0015
"	"	Ann Arbor (St)	.9007± .0100	.2647± .0018	.7930± .0060	.6827± .0033	.6600± .0016	.7254± .0029	.4450± .0013
"	"	Ann Arbor (D)	.8672± .0086	.2606± .0013	.8079± .0059	.7008± .0043	.6608± .0011	.7269± .0035	.4452± .0015
"	"	Cedarvale	.8085± .0045	.2639± .0008	.7840± .0047	.6692± .0029	.6642± .0012	.7185± .0023	.4337± .0009
"	"	Decker	.7804± .0060	.2519± .0012	.8068± .0032	.6582± .0030	.6708± .0012	.7264± .0023	.4459± .0011
"	"	Greenland	.7077± .0052	.2496± .0009	.8091± .0037	.6976± .0040	.6687± .0015	.7258± .0017	.4405± .0010
"	"	Canebrake	.8208± .0065	.2550± .0012	.8151± .0045	.6722± .0041	.6749± .0014	.7261± .0025	.4368± .0012
"	"	Asheville	.8435± .0070	.2707± .0014	.8053± .0053	.6629± .0040	.6655± .0015	.7202± .0032	.4414± .0013
eremicus	*eremicus*	Tumamoc Hill	1.0694± .0062	.2549± .0009	.8101± .0022	.8182± .0024	.6459± .0011	.7318± .0015	.4634± .0008
"	"	Tortollito Mts.	1.0347± .0055	.2488± .0009	.8091± .0029	.8062± .0030	.6482± .0011	.7296± .0018	.4610± .0008
"	"	Pima Canyon	1.0637± .0063	.2507± .0008	.8350± .0026	.8223± .0033	.6452± .0012	.7301± .0019	.4601± .0009
"	"	1000-Palm Canyon	1.0710± .0083	.2520± .0009	.7996± .0024	.8345± .0037	.6532± .0009	.7365± .0028	.4614± .0007

used were not random samples of the same homogeneous population, weighted averages of the ratios, means, and coefficients of variation were not used. The unweighted averages were calculated by using the following formula:

$$\frac{\Sigma A}{n} \pm \sqrt{\frac{\Sigma A^2 - (\Sigma A)^2/n}{n(n-1)}}$$

in which A represents the values to be averaged.

The average ratios are shown in table 4, and the discussion of ratios will be largely confined to the data in this table. The average means of the various body measurements in table 6 also aid in the interpretations of the ratios.

The tail/body ratios of the different species and subspecies differ greatly. Within the species *P. maniculatus*, the subspecies *P. m. artemisiae*, *P. m. gambelii*, and *P. m. rubidus* have significantly higher ratios than the other mice in this species (table 4). This is due mostly to their longer tails, all three being at times tree-inhabiting forms, while the other five subspecies are mostly nonarboreal. The body lengths of these three subspecies differ very little from those of the nonarboreal forms (table 6). The average tail/body ratios of the three species *P. maniculatus*, *P. leucopus*, and *P. eremicus* are respectively $.7677 \pm .033$, $.8329 \pm .014$, and $1.0597 \pm .008$. The ratio of *P. eremicus* is significantly higher than those of the other two species, whose ratios are hardly significantly different from one another. It should be pointed out, however, that the tail/body ratio of *P. leucopus noveboracensis* is similar to that of the subspecies *P. maniculatus artemisiae*. *P. eremicus eremicus* has a very high ratio due to the fact that it has an extremely long tail with only a slightly longer body than the other species (table 6). It should be emphasized that the average ratios of these species should not be taken as typical of the whole species. In *P. maniculatus*, for example, if fewer subspecies of the nonarboreal type had been included, the ratio would be higher. *P. leucopus* is semiarboreal, and *P. eremicus* is saxicolous in habit. Each is represented here by only one subspecies.

The average skull length/body length ratios are much less variable than the tail/body ratios, although significant differences do occur. Here there is very little correlation between habit and size of ratio. For example, the semiarboreal subspecies *P. m. artemisiae* has about the same ratio (table 4) as the prairie subspecies *P. m. bairdii*. On the other hand, the saxicolous form *P. eremicus eremicus*, although it has a tail length out of all proportion to its body length, also has a shorter skull in relation to body length than any of the other species and subspecies.

In the species *P. maniculatus*, the semiarboreal subspecies *P. m. rubidus*

TABLE 4

Average ratios and their standard errors between certain measurements of several species and subspecies of Peromyscus.

SPECIES	SUBSPECIES	NO. OF STOCKS AVERAGED	TAIL / BODY	SKULL LENGTH / BODY	FEMUR / FOOT	EAR / SKULL LENGTH	MANDIBLE / SKULL LENGTH	CONDYLE-ZYGOMA / SKULL LENGTH	SKULL WIDTH / SKULL LENGTH
maniculatus	*artemisiae*	3	.8212±.044	.2615±.001	.7933±.006	.7268±.003	.6499±.003	.7126±.004	.4368±.001
"	*bairdii*	6	.6608±.043	.2624±.002	.8030±.019	.6365±.006	.6711±.002	.7301±.000	.4545±.002
"	*blandus*	2	.7470±.005	.2588±.001	.8023±.003	.7234±.022	.6601±.003	.7161±.000	.4369±.002
"	*gambelii*	1	.7941±.005	.2538±.001	.8040±.003	.7553±.003	.6550±.001	.7140±.002	.4420±.001
"	*osgoodi*	7	.7073±.035	.2572±.004	.8049±.008	.6914±.016	.6644±.002	.7171±.003	.4471±.002
"	*rubidus*	1	.9606±.007	.2607±.001	.7612±.003	.7185±.004	.6485±.001	.7161±.003	.4386±.001
"	*rufinus*	6	.7249±.034	.2648±.005	.7935±.005	.7593±.006	.6573±.001	.7129±.001	.4427±.002
"	*sonoriensis*	4	.7258±.046	.2609±.007	.8052±.004	.7121±.019	.6508±.002	.7221±.002	.3382±.008
	Species average		.7677±.033	.2600±.001	.7959±.005	.7154±.014	.6571±.003	.7176±.002	.4296±.013
leucopus	*noveborascensis*	8	.8329±.014	.2594±.002	.8045±.004	.6781±.005	.6657±.002	.7230±.002	.4406±.002
eremicus	*eremicus*	4	1.0597±.008	.2516±.001	.8134±.008	.8203±.001	.6481±.002	.7318±.002	.4617±.001
	Genus average		.8034±.039	.2591±.001	.7985±.005	.7222±.016	.6571±.002	.7196±.002	.4239±.012

tends to have a shorter femur in relation to foot length than most of the nonarboreal forms. This is shown by its small ratio (table 4). However, another semiarboreal form, *P. m. gambelii*, has a ratio almost identical with those of the nonarboreal subspecies *P. m. bairdii*, *P. m. blandus*, *P.m. osgoodi*, and *P. m. sonoriensis*. The saxicolous species *P. eremicus eremicus* tends to have a longer femur in relation to foot length than any of the other species or subspecies, although the difference is not significant in every case.

In table 4, it may readily be seen that mice of the species *P. e. eremicus* have a strikingly higher ear/skull length ratio ($.8203 \pm .001$) than any of the other forms here compared. The *eremicus* mice have exceptionally long ears. On the other hand, mice of the form *P. m. bairdii* have a smaller ratio ($.6365 \pm .006$) than mice of the other species or subspecies, due to their extremely short ears.

The mandible/skull length ratios and condyle-zygoma/skull length ratios reveal few if any differences that would add to the value of this discussion.

The skull width/skull length ratios (table 4) reveal certain interesting differences. The short-eared, short-tailed deer mouse *P. m. bairdii* has a wider skull in relation to skull length than any of the other subspecies of the species *P. maniculatus*, its ratio being $.4545 \pm .002$. This ratio is exceeded only by that of the cactus mouse, *P. eremicus eremicus*, which has a ratio of $.4617 \pm .001$.

MEANS OF THE MEASUREMENTS

The means and their standard errors of the measurements of the individual stocks of *Peromyscus* are listed in table 5. The average means of the species and subspecies were calculated from the data in table 5 and are found in table 6. Differences between these stocks in size of body parts have been reported by DICE (1932, 1933, 1938, 1939a, 1939b). No attempt is made here to analyze these means, since DICE has already done so. He has also discussed the genetic and evolutionary significance of these data (DICE 1940a, 1940b). The data are included so that the other results presented in this paper will be more intelligible, particularly the ratios.

VARIABILITY[1]

The coefficients of variation and their standard errors of the individual stocks are shown in table 7, and the averages appear in table 8.

As might be expected, different stocks of mice of the same subspecies often differ significantly from each other in variability. For example, *P. m.*

[1] Tables of standard deviations of all stocks are too extensive to reproduce, but are on file in the Editorial Office of GENETICS and will be supplied on request to persons interested.

TABLE 5

The means and their standard errors of some measurements of Peromyscus, in millimeters.

SPECIES	SUBSPECIES	LOCALITY	BODY	TAIL	FOOT	EAR	FEMUR	MANDIBLE	SKULL LENGTH	CONDYLE-ZYGOMA	SKULL WIDTH
maniculatus	*artemisiae*	Godman Springs	91.14±.556	80.05±.594	20.16±.072	17.87±.082	15.77±.086	15.75±.049	24.44±.067	17.31±.049	10.71±.028
"	"	Palouse Falls	96.64±.422	70.99±.482	20.12±.061	17.99±.077	16.17±.071	16.14±.044	24.67±.062	17.51±.041	10.81±.026
"	"	Troy	93.34±.364	79.39±.500	20.09±.090	17.53±.079	15.97±.059	15.84±.050	24.34±.069	17.54±.049	10.58±.025
"	*bairdii*	Ann Arbor	81.35±.400	57.37±.380	17.82±.069	13.66±.059	14.06±.071	14.03±.057	22.09±.067	16.17±.050	10.12±.034
"	"	Alexander	82.94±.420	60.41±.300	18.13±.083	14.28±.068	14.73±.072	15.37±.053	22.77±.087	16.58±.066	10.24±.038
"	"	Barrett	92.34±.440	58.58±.450	18.48±.060	15.01±.066	15.17±.069	15.77±.040	23.39±.057	17.06±.043	10.60±.031
"	"	Onaga	88.76±.350	55.75±.390	19.12±.059	14.88±.040	15.29±.086	15.33±.038	23.02±.060	16.79±.044	10.60±.032
"	"	Sand Point	90.18±.335	55.26±.406	18.03±.059	15.18±.058	14.62±.063	15.30±.042	23.01±.044	16.84±.037	10.55±.026
"	"	Lake Village	89.60±.386	58.60±.396	18.64±.063	14.44±.067	14.61±.072	15.86±.050	23.10±.063	16.90±.049	10.36±.033
"	*blandus*	Alamogordo	94.44±.654	71.06±.514	20.81±.069	18.32±.078	16.75±.096	16.17±.056	24.59±.078	17.57±.055	10.81±.032
"	"	White Sands	94.28±.493	69.91±.512	21.45±.072	17.25±.069	17.13±.084	16.30±.059	24.59±.073	17.65±.054	10.68±.029
"	*gambelii*	La Jolla	95.12±.330	75.44±.470	20.46±.051	18.28±.054	16.45±.050	15.84±.028	24.20±.042	17.29±.031	10.69±.019
"	*osgoodi*	Cottonwood Basin	94.66±.574	66.28±.627	20.64±.086	16.28±.085	15.97±.092	16.15±.062	24.23±.089	17.51±.063	10.77±.032
"	"	Mandan	95.38±.406	63.25±.447	19.59±.057	15.89±.065	15.88±.064	15.83±.045	23.86±.067	17.20±.048	10.76±.028
"	"	Otis	94.46±.430	63.35±.399	20.54±.058	16.91±.073	16.17±.060	16.40±.039	23.70±.059	17.60±.040	10.82±.025
"	"	Jackson	85.39±.445	76.10±.492	19.25±.080	17.99±.070	15.49±.087	15.76±.052	23.77±.083	16.88±.062	10.76±.033
"	"	Logan Canyon	93.77±.467	71.01±.612	20.46±.073	17.77±.078	16.21±.085	15.85±.049	24.21±.083	17.07±.041	10.75±.031
"	"	Taylor	96.62±.446	60.67±.455	19.33±.054	16.04±.060	16.17±.056	16.17±.047	24.39±.060	17.64±.046	10.88±.030
"	"	Gregory	94.18±.470	60.33±.420	19.40±.081	15.35±.095	16.08±.069	16.09±.058	23.98±.075	17.26±.052	10.81±.035
"	*rubidus*	Eugene	92.78±.451	83.49±.724	20.95±.063	17.38±.079	15.94±.076	15.68±.047	24.19±.074	17.33±.055	10.61±.030
"	*rufinus*	La Veta	87.50±.480	73.42±.610	20.61±.076	18.10±.108	16.14±.078	16.02±.072	24.26±.083	17.32±.057	10.78±.034
"	"	Deadman Flat	93.10±.509	61.14±.626	19.88±.077	18.92±.078	15.93±.079	15.83±.055	24.10±.085	17.17±.058	10.70±.026
"	"	Little Spring	94.57±.388	61.66±.351	19.71±.064	18.36±.064	15.68±.062	15.90±.037	24.25±.056	17.32±.040	10.77±.022
"	"	Ice Caves	96.97±.461	65.46±.607	20.08±.084	18.87±.094	16.36±.103	16.10±.069	24.50±.075	17.56±.058	10.94±.027
"	"	Bonito Canyon	92.39±.488	66.30±.545	20.30±.104	18.03±.090	16.02±.078	15.96±.040	24.29±.090	17.31±.060	10.80±.035
"	"	Cloudcroft	86.48±.402	70.34±.486	20.21±.072	18.38±.070	15.71±.070	16.08±.049	24.33±.078	17.26±.043	10.78±.026
"	*sonoriensis*	Miler Canyon	96.20±.580	61.55±.464	20.38±.074	16.94±.079	16.57±.082	15.93±.056	24.34±.084	17.62±.055	10.77±.030
"	"	Winslow	93.38±.375	61.09±.374	19.75±.071	17.80±.122	15.80±.088	15.67±.061	23.94±.071	17.16±.057	10.67±.040
"	"	San Felipe	95.92±.524	75.11±.638	20.61±.057	17.92±.073	16.41±.078	15.54±.051	24.12±.083	17.40±.053	10.70±.025
"	"	Victorville	88.81±.414	73.38±.472	19.29±.068	16.69±.067	15.68±.079	15.15±.063	25.05±.084	16.96±.057	10.54±.035
leucopus	*noveboracensis*	Ann Arbor (W)	95.77±.380	80.84±.485	20.56±.058	16.90±.054	16.76±.066	16.40±.044	24.81±.058	17.73±.044	10.82±.027
"	"	Ann Arbor (St)	92.33±.758	83.16±.687	20.62±.083	16.64±.064	16.36±.102	16.08±.063	24.38±.072	17.62±.054	10.86±.029
"	"	Ann Arbor (D)	92.36±.465	80.09±.791	20.22±.101	16.80±.082	16.34±.075	15.86±.066	23.98±.087	17.40±.071	10.68±.046
"	"	Cedarvale	93.49±.376	75.58±.443	20.60±.072	16.54±.061	16.15±.056	16.42±.045	24.72±.059	17.76±.046	10.72±.024
"	"	Decker	98.82±.510	77.12±.630	20.91±.078	16.36±.059	16.87±.084	16.64±.060	24.86±.070	17.92±.050	11.06±.025
"	"	Greenland	100.02±.351	79.78±.554	20.84±.059	17.44±.091	16.86±.071	16.73±.045	25.00±.064	18.15±.044	11.00±.078
"	"	Canebrake	98.08±.595	80.50±.661	20.63±.081	16.81±.091	16.81±.090	16.91±.057	25.01±.069	18.21±.052	10.97±.029
"	"	Asheville	90.20±.459	76.14±.664	20.13±.065	16.18±.087	16.21±.077	16.23±.048	24.41±.071	17.58±.068	10.78±.033
eremicus	*eremicus*	Tumamoc Hill	93.83±.351	100.34±.611	20.29±.054	19.46±.052	16.44±.046	15.36±.033	23.79±.044	17.43±.031	11.03±.017
"	"	Tortollito Mts.	98.91±.389	102.34±.480	20.96±.059	19.75±.062	16.96±.059	15.88±.038	24.50±.052	17.87±.033	11.31±.022
"	"	Pima Canyon	97.33±.375	103.54±.552	20.56±.069	19.96±.066	17.17±.056	15.66±.041	24.27±.057	17.71±.038	11.17±.022
"	"	1000-Palm Canyon	95.76±.436	103.04±.684	20.48±.051	20.14±.081	16.38±.058	15.76±.035	23.13±.045	17.76±.029	11.14±.020.

TABLE 6

The average means and their standard errors of some measurements of several species and subspecies of Peromyscus.

SPECIES	SUBSPECIES	NO. OF STOCKS AVERAGED	BODY	TAIL	FOOT	EAR	FEMUR	MANDIBLE	SKULL LENGTH	CONDYLE-ZYGOMA	SKULL WIDTH
maniculatus	*artemisiae*	3	93.71±1.60	76.81±2.92	20.12±0.02	17.80±0.19	15.97±0.12	15.91±0.12	24.48±0.10	17.45±0.07	10.70±0.07
"	*bairdii*	6	87.53±1.78	57.66±0.79	18.37±0.19	14.68±0.23	14.75±0.18	15.28±0.27	22.90±0.18	16.72±0.13	10.41±0.08
"	*blandus*	2	94.36±0.08	70.48±0.58	21.13±0.32	17.78±0.29	16.94±0.19	16.24±0.06	24.59±0.00	17.61±0.04	10.74±0.06
"	*gambelii*	1	95.12±0.33	75.44±0.47	20.46±0.05	18.28±0.05	16.45±0.05	15.84±0.03	24.20±0.04	17.29±0.03	10.69±0.02
"	*osgoodi*	7	93.49±1.40	65.86±2.19	19.89±0.24	16.60±0.37	16.00±0.10	16.04±0.09	24.02±0.10	17.31±0.11	10.79±0.02
"	*rubidus*	1	92.78±0.45	83.49±0.72	20.95±0.06	17.38±0.08	15.94±0.08	15.68±0.05	24.19±0.07	17.33±0.05	10.61±0.03
"	*rufinus*	6	91.84±1.67	66.39±1.96	20.13±0.13	18.44±0.15	15.97±0.19	15.98±0.04	24.29±0.05	17.32±0.05	10.80±0.03
"	*sonoriensis*	4	93.58±1.71	67.78±3.75	20.01±0.30	17.34±0.31	16.12±0.22	15.57±0.16	24.36±0.24	17.28±0.14	10.67±0.05
	Species average		92.80±0.83	70.49±2.82	20.13±0.30	17.29±0.42	16.02±0.22	15.82±0.11	24.13±0.19	17.29±0.09	10.68±0.04
leucopus	*noveboracensis*	8	95.14±1.26	79.15±0.92	20.56±0.03	16.71±0.14	16.54±0.11	16.41±0.12	24.65±0.13	17.80±0.10	10.86±0.05
eremicus	*eremicus*	4	96.46±1.09	102.32±0.70	20.57±0.14	19.83±0.15	16.74±0.10	15.67±0.11	24.17±0.15	17.69±0.09	11.16±0.06
	Genus average		93.40±0.77	74.54±3.90	20.22±0.24	17.48±0.43	16.14±0.19	15.86±0.10	24.18±0.16	17.38±0.09	10.74±0.06

TABLE 7

Coefficients of variation and their standard errors for certain measurements of Peromyscus.

SPECIES	SUBSPECIES	LOCALITY	BODY	TAIL	FOOT	FEMUR	MANDIBLE	SKULL LENGTH	CONDYLE-ZYGOMA	SKULL WIDTH
maniculatus	*artemisiae*	Godman Springs	4.95±.431	6.03±.525	2.78±.254	5.33±.486	2.49±.222	2.26±.195	2.26±.201	2.10±.183
"	"	Palouse Falls	4.05±.309	6.30±.480	2.80±.215	4.03±.309	2.51±.195	2.21±.179	2.15±.166	2.18±.169
"	"	Troy	3.36±.276	5.41±.445	3.56±.317	2.92±.260	2.39±.224	2.07±.199	2.13±.198	1.79±.165
"	*bairdii*	Ann Arbor	5.02±.344	6.74±.463	3.76±.274	4.87±.355	3.63±.287	2.72±.212	2.84±.220	3.02±.239
"	"	Alexander	4.96±.356	4.97±.356	3.93±.322	4.19±.344	2.80±.251	3.05±.272	3.18±.284	2.84±.264
"	"	Barrett	4.64±.350	7.24±.545	3.16±.242	3.34±.256	2.15±.172	2.22±.175	2.29±.181	2.61±.210
"	"	Onaga	3.60±.276	6.46±.496	2.75±.218	5.04±.390	2.21±.175	2.35±.187	2.33±.184	2.74±.216
"	"	Sand Point	3.37±.263	6.66±.520	2.88±.231	3.81±.305	2.35±.195	1.67±.135	1.93±.155	2.08±.172
"	"	Lake Village	3.71±.297	5.80±.465	2.89±.238	4.24±.349	2.61±.221	2.26±.191	2.44±.206	2.62±.223
"	*blandus*	Alamagordo	7.16±.490	7.49±.510	3.32±.253	5.30±.405	3.19±.245	2.98±.220	2.95±.222	2.78±.213
"	"	White Sands	5.20±.370	7.29±.518	3.09±.280	3.82±.346	2.71±.254	2.29±.211	2.36±.217	2.08±.193
"	*gambelii*	La Jolla	4.23±.234	8.06±.445	3.23±.181	3.80±.213	2.18±.127	2.01±.116	2.02±.117	2.13±.123
"	*osgoodi*	Cottonwood Basin	4.24±.429	6.62±.669	2.99±.293	4.15±.407	2.75±.270	2.58±.261	2.58±.253	2.17±.213
"	"	Mandan	4.17±.301	6.92±.499	2.86±.208	3.83±.278	2.79±.203	2.69±.197	2.73±.198	2.54±.184
"	"	Otis	4.44±.322	6.13±.445	2.74±.200	3.57±.260	2.33±.168	2.42±.174	2.25±.162	2.25±.162
"	"	Jackson	5.57±.369	6.91±.458	3.44±.295	4.61±.395	2.77±.234	2.92±.247	3.10±.262	2.56±.218
"	"	Logan Canyon	3.95±.356	6.84±.615	2.79±.253	4.12±.373	2.49±.220	2.72±.245	1.92±.173	2.27±.204
"	"	Taylor	4.18±.326	6.79±.530	2.50±.198	3.10±.245	2.60±.204	2.20±.173	2.35±.185	2.51±.197
"	"	Gregory	4.84±.352	6.75±.492	4.01±.296	4.13±.304	3.46±.255	2.96±.222	2.93±.220	3.12±.229
"	*rubidus*	Eugene	5.13±.425	7.40±.613	2.88±.211	4.60±.338	2.92±.215	2.92±.215	3.10±.222	2.72±.200
"	*rufinus*	La Veta	4.84±.388	7.36±.589	3.08±.260	4.04±.342	3.47±.317	2.45±.226	2.56±.234	2.44±.224
"	"	Deadman Flat (D)	5.25±.428	8.86±.723	3.52±.274	4.48±.350	3.25±.248	3.24±.250	2.96±.237	2.14±.170
"	"	Little Spring	4.40±.290	6.11±.402	3.38±.230	4.12±.280	2.38±.165	2.38±.161	2.35±.164	2.02±.145
"	"	Ice Caves	3.59±.336	7.00±.655	3.11±.296	4.67±.445	3.30±.304	2.44±.216	2.70±.235	1.86±.173
"	"	Bonito Canyon	4.26±.374	6.63±.582	3.75±.361	3.56±.343	2.12±.179	2.69±.264	2.74±.244	2.38±.229
"	"	Cloudcroft	4.88±.329	7.24±.488	3.64±.251	4.57±.315	2.93±.214	2.93±.228	2.51±.177	2.27±.168
"	*sonoriensis*	Miller Canyon	5.18±.426	6.48±.532	2.97±.256	4.06±.350	3.70±.247	2.96±.245	2.70±.222	2.31±.194
"	"	Winslow	3.09±.284	4.70±.432	2.69±.254	4.16±.393	2.93±.274	2.23±.209	2.47±.234	2.78±.268
"	"	San Felipe	4.83±.387	7.50±.600	2.63±.196	4.51±.336	3.06±.230	3.03±.243	2.87±.214	2.11±.164
"	"	Victorville	3.64±.330	5.02±.455	2.72±.250	3.88±.357	3.13±.293	2.60±.237	2.62±.239	2.51±.235
leucopus	*noveboracensis*	Ann Arbor (W)	3.94±.250	5.95±.423	2.71±.197	3.83±.286	2.59±.189	2.10±.153	2.43±.176	2.42±.178
"	"	Ann Arbor (St)	5.63±.581	5.66±.584	2.78±.281	4.33±.442	2.73±.278	2.12±.210	2.03±.216	1.96±.190
"	"	Ann Arbor (D)	3.77±.366	7.39±.699	3.60±.353	3.30±.324	2.96±.296	2.61±.256	2.96±.288	3.10±.307
"	"	Cedarvale	3.71±.285	5.41±.415	3.18±.247	3.16±.246	2.51±.194	2.17±.167	2.38±.184	2.07±.160
"	"	Decker	4.33±.362	6.90±.579	3.40±.262	4.50±.354	2.94±.256	2.53±.200	2.36±.198	1.94±.158
"	"	Greenland	3.23±.248	6.40±.491	2.57±.202	3.80±.298	2.27±.190	2.14±.182	2.07±.172	2.23±.191
"	"	Canebrake	4.78±.432	6.46±.582	3.24±.278	4.42±.379	2.79±.239	2.29±.195	2.43±.200	2.16±.185
"	"	Asheville	4.14±.360	7.09±.618	2.54±.230	3.72±.337	2.28±.210	2.23±.203	2.97±.271	2.40±.216
eremicus	*eremicus*	Tumamoc Hill	3.70±.264	6.02±.430	2.97±.189	3.12±.198	2.35±.153	2.04±.123	1.98±.127	1.70±.110
"	"	Tortollitu Mts.	4.03±.278	4.80±.331	2.76±.199	3.41±.246	2.34±.171	2.05±.150	1.83±.133	1.91±.139
"	"	Pima Canyon	3.53±.272	4.89±.378	3.21±.238	3.10±.230	2.38±.184	2.14±.166	1.98±.154	1.84±.142
"	"	1000-Palm Canyon	4.36±.360	5.67±.469	2.47±.176	3.51±.250	2.01±.142	1.85±.132	1.64±.116	1.75±.124

TABLE 8

Average coefficients of variation and their standard errors for certain measurements of Peromyscus.

SPECIES	SUBSPECIES	NO. OF STOCKS AVERAGED	BODY	TAIL	FOOT	FEMUR	MANDIBLE	SKULL LENGTH	CONDYLE-ZYGOMA	SKULL WIDTH
maniculatus	*artemisiae*	3	4.12±.46	5.91±.26	3.05±.26	4.09±.70	2.46±.04	2.18±.06	2.18±.04	2.02±.12
"	*bairdii*	6	4.22±.30	6.31±.33	3.23±.20	4.25±.26	2.62±.22	2.38±.19	2.50±.18	2.65±.13
"	*blandus*	2	6.18±.98	7.39±.10	3.20±.12	4.56±.74	2.95±.24	2.64±.34	2.66±.30	2.43±.35
"	*gambelii*	1	4.23±.23	8.06±.44	3.23±.18	3.80±.21	2.18±.13	2.01±.12	2.02±.12	2.13±.12
"	*osgoodi*	7	4.48±.21	6.71±.33	3.05±.19	3.93±.18	2.74±.13	2.64±.10	2.55±.01	2.49±.12
"	*rubidus*	1	5.13±.42	7.40±.61	2.88±.21	4.60±.34	2.92±.22	2.92±.21	3.10±.22	2.72±.20
"	*rufinus*	6	4.54±.24	7.20±.38	3.41±.11	4.24±.17	2.91±.22	2.69±.14	2.64±.09	2.18±.09
"	*sonoriensis*	4	4.18±.49	5.92±.65	2.75±.07	4.15±.13	3.05±.04	2.70±.18	2.66±.08	2.43±.14
	Species average		4.64±.25	6.86±.28	3.10±.08	4.20±.10	2.73±.10	2.52±.11	2.54±.12	2.38±.09
leucopus	*noveboracensis*	8	4.19±.26	6.41±.25	3.00±.14	3.89±.18	2.63±.10	2.27±.07	2.45±.12	2.28±.13
eremicus	*eremicus*	4	3.90±.58	5.34±.30	2.85±.16	3.28±.10	2.27±.09	2.02±.11	1.86±.08	1.80±.05
	Genus average		4.52±.21	6.66±.27	3.06±.06	4.08±.12	2.67±.09	2.44±.10	2.46±.04	2.31±.09

bairdii from Alexander have a coefficient of variation for tail length of 4.97 ± .356 percent, while those from Barrett have a variation of 7.24 ± .545v. The difference of 2.27 is 3.47 times its standard error and is, therefore, significant. Other significant differences occur not only in tail length but also in the variation of the other body parts. It does not seem worth while to point out these differences here.

Not only the individual stocks of mice, but the subspecies and species differ in the variation of the body parts (table 8). However, it is of more importance, perhaps, to note which parts of the body are the most variable. We find that tail length is more variable than the other parts, varying from 5.34 ± .30 in *P. eremicus eremicus* to 8.06 ± .44 in *P. m. gambelii*. *P. m. gambelii* and *P. m. rubidus* are represented by only one stock each, so it is hardly fair to compare them with subspecies having more stocks. The arrangement of the body parts in order of their decreasing percentage of variation is: tail (6.66 ± .27), body (4.52 ± .21), femur (4.08 ± .12), foot (3.06 ± .06), mandible (2.67 ± .09), condyle-zygoma (2.46 ± .04), skull length (2.44 ± .10), and skull width (2.31 ± .09).

A comparison of the variation of the three species *P. maniculatus*, *P. leucopus*, and *P. eremicus* is scarcely permissible, because *P. maniculatus* is represented here by eight subspecies while each of the other two species is represented by only one subspecies. It is interesting to note, however, that *P. eremicus eremicus* is less variable in all the body measurements than is *P. leucopus noveborascensis*, although the differences are not significant in every case.

Differences in the variation of different stocks is not surprising in view of the fact that some stocks originated from the breeding of only two or three pairs of mice while others were the offspring of many pairs. Many of the differences are undoubtedly due to the possession of differing groups of hereditary factors. The greater apparent variation in tail length may be due to the fact that the tail is relatively long, and slight variations in it are more easily measured. On the other hand, a mouse with extreme variation in tail length would conceivably be less handicapped than one with extreme variations in skull or body, parts which house the vital organs. Extreme variations of the latter type might tend to be eliminated because of the handicaps they would impose upon the individuals possessing them.

As a whole, the small size of most of these coefficients of variation indicates that sufficiently large samples of the various mouse populations were studied.

DISCUSSION

The question of whether the great differences in body proportions in mice of the genus Peromyscus are due to heterogonic growth or to special

size factors is well worth considering. If body size were due exclusively to general size factors in Peromyscus, it would be possible with heterogonic growth to account for the great differences in body proportion found within the genus. However, if general body size were due exclusively to general size factors, we would expect to find perfect correlations between the measurements of the parts. The relatively small size of the correlations between the body measurements demonstrates conclusively that general size factors together with heterogonic growth can account for only a part of the differences in body size between races and species of these mice. Fully half of these differences appear to be due to special size factors (genetic or environmental).

The present analysis, however, cannot be regarded as a critical study of size inheritance. The relative importance of general and special size factors in growth and the question as to whether size factors reside in the chromosomes or in the cytoplasm can be determined with certainty only by appropriate crosses and by a study of growth in all its stages. Those interested in such studies should refer to CASTLE (1929, 1931, 1932, and 1934); CASTLE, GATES, and REED (1936); CASTLE, GATES, REED, and LAW (1936); GREEN (1931, 1933, 1935); GREGORY and CASTLE (1931); SUMNER (1923, 1932), and SVIHLA (1932).

CONCLUSIONS

Ratios between body measurements indicate that the species and subspecies of mice of the genus Peromyscus differ greatly in body proportion.

Tail length shows more variation than other measurements of the body.

Body size and proportion in Peromyscus cannot be attributed primarily to general size factors and heterogonic growth.

Differences in tail, body, foot, femur, mandible, skull length, condylezygoma, and skull width are due primarily to special size factors (genetic and developmental).

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STERILE AND FERTILE AMPHIDIPLOIDS: THEIR POSSIBLE RELATION TO THE ORIGIN OF NICOTIANA TABACUM[1]

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INTRODUCTION

THE discovery by ROSENBERG (1909) of the "Drosera scheme" of pairing and its subsequent use by other investigators as an indicator of chromosome similarity, and hence of relationship between species, has made it possible to conjecture on the origin of species which show this behavior when crossed with certain related species. One of the earliest cases in which knowledge of the "Drosera scheme" was applied to a problem of practical as well as theoretical import is that of the origin of *Nicotiana Tabacum*. It was shown that *N. Tabacum* (2n = 48) exhibited "Drosera scheme" with *N. sylvestris* (2n = 24) and with *N. tomentosa* (2n = 24) (GOODSPEED and CLAUSEN 1927, 1928). At about the same time the chromosome behavior of haploid *N. Tabacum* (CLAUSEN and MANN 1924; CHIPMAN and GOODSPEED 1927; LAMMERTS 1934) lent support to the view that the chromosome complement of *N. Tabacum* consisted of two sets, one from *N. sylvestris* and one from *N. tomentosa*. It was also shown that the F_1 hybrid *N. sylvestris* × *N. tomentosa* showed only a slight amount of chromosome pairing at meiosis (GOODSPEED and CLAUSEN 1928; GOODSPEED 1934).

It was soon recognized that *N. tomentosiformis*, a species closely related to *N. tomentosa*, could be substituted for the latter species in the *N. sylvestris–N. tomentosa–N. Tabacum* triangle (CLAUSEN 1932; KOSTOFF 1938). Other evidence in support of the hypothesis of the origin of *N. Tabacum* was sought. BRIEGER (1928b) showed that a 48 chromosome derivative from the backcross (*N. Tabacum* × *N. tomentosiformis*) × (*N. Tabacum*) showed 24 pairs at meiosis. This proved that the 12 pairs of chromosomes in the F_1 hybrid *N. Tabacum* × *N. tomentosiformis* consisted of *Tabacum* and *tomentosiformis* homologues. Similarly, it was shown that plants arising from unreduced gametes in the backcross (*N. Tabacum* × *N. tomentosiformis*) × (*N. sylvestris*) showed mostly 24 pairs plus one or two univalents at meiosis (BEISSER 1934) thus showing that the *N. sylvestris* set had its homologues in the *sylvestris* set of *N. Tabacum*. *N. triplex*, reported by KOSTOFF (1933), which is similar in its genetic constitution and chromo-

[1] Part of a dissertation submitted in partial satisfaction for the degree of Doctor of Philosophy at the University of California.

somal behavior to the plants of BEISSER mentioned above, was made by pollinating *N. Tabacum* with pollen from the extremely sterile F_1 *N. sylvestris*×*N. tomentosiformis*. BRIEGER thought *N. Tabacum* carried the recessive alleles, or completely lacked some of the genes, of *N. tomentosiformis* (curvature of the flower, projecting style and anthers).

Evidence of the correspondence of gene loci of the parental species with those of *N. Tabacum* was furnished by CLAUSEN (1932). Employing monosomics of *N. Tabacum*, CLAUSEN has shown that the P chromosome of *N. sylvestris* is similar in its genetic constitution to that of the *N. Tabacum* homologue, and the loci of factors for pink flower color and broad versus constricted leaf base correspond in the two species. There is also evidence that certain factors are present in duplicate in *N. Tabacum* and that the dominant alleles of these factors are present in one or the other of the parental species. This is what one would expect if *N. Tabacum* were of amphidiploid origin. Using the monosomic technique, chromosomes may also be assigned to one or the other of the parental genomes. The above body of evidence left little doubt concerning the origin of *N. Tabacum*.

There remained but the actual synthesis of *N. Tabacum* from its putative ancestors. An attempt at this synthesis was made by KOSTOFF (1938). This author, using the same strains of the parental species as the writer, obtained some fertile amphidiploids *N. sylvestris-tomentosiformis* by the series of crosses F_1 [(*N. sylvestris*×*N. tomentosiformis*)×*N. sylvestris*]× (*N. tomentosiformis*). This synthesis was possible due to unreduced gametes in the F_1 *N. sylvestris*×*N. tomentosiformis* and in the 36 chromosome plants F_1 (*N. sylvestris*×*N. tomentosiformis*)×*N. sylvestris*. This method employed by KOSTOFF, however, is inadequate to produce amphidiploids without altering the constituent chromosomes themselves. The two 36 chromosome plants he obtained after numerous attempts over a period of years were, as expected, much more fertile than the extremely sterile F_1 hybrid. The two-three bivalents which are usually found at first metaphase in this hybrid lead to exchange between *N. sylvestris* and *N. tomentosiformis* chromosomes; further changes in the chromosomes may also be introduced in the next step of the synthesis. The two 48 chromosome plants which he finally obtained were described by him as having been "almost fully fertile and were very much *N. Tabacum* like. Morphologically they were not quite alike. . . ." It must be added that their progeny also was not uniform and that some more and other less fertile plants segregated, although a stable type was established after a few generations of selfing. It is clear, then, that KOSTOFF's plants are numerically but not structurally amphidiploids. Plants of the same amphidiploid made by the callus method (GREENLEAF 1938) from F_1 hybrids *N. sylvestris*×*N. tomentosiformis* are completely female sterile, even though their pollen is more than

90 percent good. They are highly uniform, since the callus method of inducing polyploids avoids chromosomal alterations. They differ from the KOSTOFF amphidiploid in habit (the latter closely resembles *N. Tabacum*) and in features of the flower, but the kinship is also clear. The writer has made the amphidiploid *N. tomentosiformis-sylvestris* in the same manner. It is also completely female sterile even though its pollen is as good as that of the reciprocal amphidiploid.

Three additional amphidiploids have been made by the callus method. They are: *N. sylvestris-tomentosa*, *N. sylvestris-Setchellii*, and *N. glutinosa-tomentosa*. The last one is not concerned in the origin of *N. Tabacum*. These three amphidiploids like *N. sylvestris-tomentosiformis* (4n) Gr[2] are female sterile and male fertile.

Since several forms or races exist of some of the species employed, and since the writer has found that some give sterile and others fertile amphidiploids, the races and species used will be briefly mentioned. The sterile, callus-induced amphidiploids already cited involve the long inbred lines of *N. sylvestris* Speg. & Comes U.C.B.G. 07–69,[3] (Argentina); *N. glutinosa* L. U.C.B.G. 07–79, (Peru); *N. tomentosa* Ruiz & Pavon U.C.B.G. 08–193, (Brazil and Peru); and *N. tomentosiformis* Goodsp. U.C.B.G. 25–12, (Amazon Basin, Peru). *N. tomentosiformis* has been used under the name *N. Rusbyi* by some workers. *N. Setchellii*[4] which is related to *N. tomentosa* has recently been introduced from Peru. A brief examination of P.M.C. of the hybrid *N. Tabacum*×*N. Setchellii* confirmed the inference made on morphological grounds that this species is also among those which give the "Drosera scheme" of pairing with *N. Tabacum*. Later studies of chromosome pairing and of pollen abortion in the F_1 hybrid *N. Tabacum*×*N. sylvestris-Setchellii* (4n) have shown, however, that the chromosome set of this species is not so similar to the *tomentosa* set of *N. Tabacum* as is that of *N. tomentosa* and of *N. tomentosiformis*. This is also evident from the F_1 hybrids *N. tomentosiformis*×*N. tomentosa* and *N. tomentosiformis*×*N. Setchellii* which have 98 and 39 percent of good pollen, respectively. The former is highly female fertile, the latter much less.

A number of races of *N. tomentosa* are known which have recently been introduced from South America. It was thought that perhaps some of these would give fertile amphidiploids with *N. sylvestris*, for it is possible that the original *N. tomentosa* U.C.B.G. 08–193 and *N. tomentosiformis*

[2] The female sterile, male fertile amphidiploid of GREENLEAF as contrasted with the female fertile, male fertile one of KOSTOFF.

[3] University of California Botanical Garden accession number. The first number refers to the year when received.

[4] A new species of Nicotiana collected by MR. BAYNE BEAUCHAMP near Chachapoyas, Dpto. Amazonas, Peru, in 1937, named and to be described by T. H. GOODSPEED in UNIVERSITY OF CALIFORNIA Publications in Botany.

(only one race is known of this species) might have been unknowingly selected to give the results obtained. By the callus method (loc. cit.) autotetraploids were made of four very distinct races: *N. tomentosa*—Acomayo[5] U.C.B.G. 36–40, (Dpto. Huanuco, Peru); *N. tomentosa*—Machu Picchu U.C.B.G. 36–165, (Dpto. Cuzco, Peru); *N. tomentosa*—Pincos U.C.B.G. 36–32, (Dpto. Apurimac, Peru); and *N. tomentosa*—Ticempaya U.C.B.G. 36–59, (Dpto. La Paz, Bolivia). Likewise, autotetraploids of *N. sylvestris* U.C.B.G. 07–69 and of *N. tomentosiformis* were made. By crossing *N. sylvestris* (4n) with these several autotetraploid races of *N. tomentosa* and with autotetraploid *N. tomentosiformis* the corresponding amphidiploids were secured.[6] Two of these amphidiploid lines, *N. sylvestris* (4n)×*N. tomentosa*—Acomayo (4n) (39115) and *N. sylvestris* (4n)×*N. tomentosa*—Machu Picchu (4n) (39113), contain partially fertile plants (table 3). As may be seen from the table, 16 out of the 20 plants of 39113 which flowered had one or more seed pods. That not all the plants which flowered set pods is not surprising when one considers the low fertility of these amphidiploids. Two counts made from controlled cross-pollinations within the same lines showed five percent (11/223) good seeds in 39115P18 and two percent (4/166) in 39113P10. A number of controlled intralinear-pollinations made on three plants of each line resulted in a variation in the number of seeds per pod. Some plants appear to be potentially rather fertile, occasionally producing as many as 500 seeds in one pod, but usually producing only 50–200 per pod. Since hundreds of ovules are present in the ovary, plants with a low percentage of potentially good ovules produce a great many viable seeds even in one pod. That many of the plants which have flowered produce only one or two capsules per inflorescence (about 100(?) flowers) may explain why other plants with fewer flowers fail to set any pods when left to open-pollinate. They may be partially fertile, although probably not so fertile as their sister plants. None of the 17 plants of 39124 which flowered set any pods whatever on chance pollination in the field. Two plants of this line, when crossed with 39115, set seed. It appears, then, that the line 39124, although less fertile than 39113 and 39115, contains potentially fertile plants. Studies of embryo sac development of plants which have set pods and of others which have not will show if there is a difference in potential fertility. Fifty-four plants of *N. sylvestris* (4n)×*N. tomentosiformis* (4n) were as sterile as the corresponding callus-induced amphidiploid.

In order to discover the cytological phenomena which accompany female sterility, a microscopic examination of the ovaries of *N. sylvestris-tomentosa* (4n), *N. sylvestris-tomentosiformis* (4n) Gr, and *N. tomentosiformis-syl-*

[5] The racial designations are according to locality where collected.

[6] The autotetraploid callus shoots themselves were used in making the crosses.

vestris (4n) was made. This was followed by similar studies of *N. sylvestris-Setchellii* (4n) and *N. glutinosa-tomentosa* (4n) and of the F_1 hybrids *N. sylvestris-tomentosiformis* (4n) Kostoff×*ditto* Gr, *N. Tabacum*×*N. sylvestris-tomentosiformis* (4n) Gr, *N. Tabacum*×*N. sylvestris-Setchellii* (4n), and *N. sylvestris*×*N. tomentosiformis*. Two female sterile F_2 segregates from the F_1 *N. Tabacum*×*N. sylvestris-tomentosiformis* (4n) Gr were also

TABLE 1

Megasporogenesis—number of ovules studied.

	SIZE OF BUD IN CM.	2.1–2.9			3.1–3.5			3.5–3.8		4.1–4.6	4.6–4.8	4.8–5.1
		—	MI	MII	—	MI	MII	—	MI			
1	*Nicotiana Tabacum*							10		13	11	
2	*N. sylvestris-tomentosa* (4n)	9	7	2	17	3		26	1	5	9	
3	*N. sylvestris-tomentosiformis* (4n) Gr.				6							
4	*N. tomentosiformis-sylvestris* (4n)							25				
5	*N. sylvestris-Setchellii* (4n)				7			20				
6	*N. glutinosa-tomentosa* (4n)	17										
7	F_1 *N. sylvestris*× *N. tomentosiformis*											35
8	F_1 [*N. sylvestris-tomentosiformis* (4n) Kostoff] ×(ditto Gr)									29		
9	F_1 (*N. Tabacum*)×[*N. sylvestris-tomentosiformis* (4n) Gr]									14		
10	F_1 (*N. Tabacum*)×[*N. sylvestris-Setchellii* (4n)]				5	13	3					
11	F_2 (*N. Tabacum*)×[*N. sylvestris-tomentosiformis* (4n) Gr] plant 11							27				12
12	F_2 (*N. Tabacum*)×[*N. sylvestris-tomentosiformis* (4n) Gr] plant 95											10

investigated. For the comparison of normal ovule development *N. Tabacum purpurea* was studied. (*N. Tabacum purpurea* was also used in all the crosses involving *N. Tabacum* reported in this paper.)

The stages of megasporogenesis and embryo sac development of all plants examined are summarized in tables 1 and 2. These studies showed that megasporogenesis in the completely female sterile amphidiploids *N. sylvestris-tomentosa*, *N. sylvestris-tomentosiformis*, and *N. tomentosiformis-sylvestris* is regular but that development of the embryo sacs is arrested in

TABLE 2

Embryo sac development—number of ovules studied.

	5.4–5.6 CM. BUDS	6.0–6.7 CM. BUDS	FLOWER JUST BEGINNING TO OPEN	FLOWER OPEN ANTHERS CLOSED	Flower fully open					24 HRS. AFTER POLLINATION	32 HOURS	44 HOURS	47–49 hours						56 HOURS	64 HOURS	71–76 HOURS	93–96 HOURS	120 HOURS	144–147 HRS.	COROLLA DRIED	168 HOURS	192 HOURS	216 HOURS AFTER POLLINATION
					— — —	1 N.E.S.	2 N.E.S.	4 N.E.S.	8 N.E.S.	COLLAPSED DIS.			— — —	COLLAPSED INDISTING.	1 N.E.S. DIS.	2 N.E.S. DIS.	4 N.E.S. DIS.	4 N.E.S. HEALTHY	8 N.E.S. DIS.	8 N.E.S. HEALTHY									
1 *Nicotiana Tabacum*			2				1	1	2										9				many					
2 *N. sylvestris-tomentosa* (4n)	4	13								9	8	5	many		6	5				13	14	12	31	14	6			5
3 *N. sylvestris-tomentosiformis* (4n) Gr		3								14			many		2	3						14	7		4			4
4 *N. tomentosiformis-sylvestris* (4n)							3	1		1			14		10									3+ many dis.				
5 *N. sylvestris-Setchellii* (4n)						1	6	7	1	2					9	4		1				9	4	6	4		25	
6 *N. glutinosa-tomentosa* (4n)																						15	1		28			
7 F_1 *N. sylvestris* × *N. tomentosiformis*				12	13																					10		
8 F_1 [*N. sylvestris-tomentosiformis* (4n) Kostoff] × (ditto Gr)						1		4	1	3				1	1	1	4	4	4			12		many				
9 F_1 (*N. Tabacum*) × [*N. sylvestris-tomentosiformis* (4n) Gr]							4	5	3	1												5		4				
10 F_1 (*N. Tabacum*) × [*N. sylvestris-Setchellii* (4n)]																						8	6	26				
11 F_2 (*N. Tabacum*) × [*N. sylvestris-tomentosiformis* (4n) Gr plant 11							3	6						2		2	8							many dis.				
12 F_2 (*N. Tabacum*) × [*N. sylvestris-tomentosiformis* (4n) Gr plant 95							1	10		2																	many dis.	

either the two- or four-nucleate stage. This also occurs in the amphidiploid *N. glutinosa-tomentosa* which has less regular meiosis. *N. sylvestris-Setchellii* (4n) exhibits somewhat different behavior. In it, two-, four-, and eight-nucleate embryo sacs occur in various stages of disintegration four days and later after self-pollination. This amphidiploid is also completely or almost completely sterile. Sterility is not due to failure of embryo sac development alone, for pollen tubes fail to reach the ovary. A similar cytological examination of the hybrids between *N. Tabacum* and two of the sterile amphidiploids and of the F_1 *N. sylvestris-tomentosiformis* (4n) Kostoff×*ditto* Gr revealed that they are only partially fertile. This indi-

TABLE 3

*Fertility of amphidiploids from crosses between autotetraploids.**

PEDIGREE	NOT FLOWERING	NUMBER OF PODS PER PLANT											
		None	1	2	3	4	5	7	10	11	15	20	28
39113	20	4	6	1	1	4			1	2			1
39114		54											
39115		16	6	2	2	1	1	1			1	1	
39117		2											
39124	20	17											

Key:

39113—*N. sylvestris* (4n)×*N. tomentosa*—Machu Picchu (4n)
39114—*N. sylvestris* (4n)×*N. tomentosiformis* (4n)
39115—*N. sylvestris* (4n)×*N. tomentosa*—Acomayo (4n)
39117—*N. sylvestris* (4n)×*N. tomentosa*—Ticempaya (4n)
39124—*N. sylvestris* (4n)×*N tomentosa*—Pincos (4n)

* Fertility was surmised from number of pods set when plants were left to open pollinate in the field.

cates that some ovules receive viable, others inviable, combinations of genes. In each the disintegration of embryo sacs is like that of the sterile amphidiploid *N. sylvestris-tomentosa*. The same was found in the two sterile F_2 segregates.

The cytological studies of megasporogenesis and embryo sac development have led to the following hypothesis: Only those megaspores which do not carry the sterility genes can give rise to functional embryo sacs. These genes are invariably present in the megaspores of the sterile amphidiploids. They do not, however, affect the viability of the pollen.

Genetic evidence in support of the hypothesis came from an F_2 *N. Tabacum*×*N. sylvestris-tomentosiformis* (4n) Gr (table 4), which shows only three completely sterile plants in 73, and from the backcross of F_1 (*N. Tabacum*×*N. sylvestris-tomentosiformis* (4n) Gr)×*ditto* Gr, which has given about the same results as the F_2—namely, two completely sterile

TABLE 4

Fertility data.

	PLANTS VARIOUSLY FERTILE	PLANTS STERILE OR NEARLY SO	
		FEW SEEDS PER CAPSULE	PLANTS STERILE OR NEARLY SO
1	68	2	3
2	50	1	2

Key:

1—F_2 [(*N. Tabacum*)×[*N. sylvestris-tomentosiformis* (4n) Gr]]

2—Backcross [(*N. Tabacum*)×[*N. sylvestris-tomentosiformis* (4n) Gr]]×(*ditto* Gr).

plants in 53 (table 4). KOSTOFF (1933) reported similar findings. He found no sterile plants in 127 F_2 progeny of F_1 *N. triplex* plants. If the female gametophytes which receive the sterility genes do not function, this result is expected.

Similar results were obtained in the F_2 from F_1 *N. sylvestris-tomentosiformis* (4n) Kostoff×*ditto* Gr and from the reciprocal backcrosses of this F_1 hybrid to the fertile Kostoff amphidiploid and to the sterile Gr amphidiploid. All plants obtained from these crosses are fertile as expected if the hypothesis is correct. The results are summarized in table 5.

TABLE 5

Fertility data concerning gametic lethal hypothesis.

	FERTILE	NOT FLOWERING	MISSING	DEAD	TOTAL
1	28	10	10	1	49
2	31	2	1	3	37
3	25	2	3	6	36
4	34	0	5	3	42

Key:

1—F_2 [*N. sylvestris-tomentosiformis* (4n) Kostoff×*ditto* Gr]

2—F_1 [*N. sylvestris-tomentosiformis* (4n) Kostoff×*ditto* Gr] ×(*ditto* Gr)

3—[*N. sylvestris-tomentosiformis* (4n) Kostoff]×[F_1 *ditto* Kostoff×*ditto* Gr]

4—[F_1 *N. sylvestris tomentosiformis* (4n) Kostoff×*ditto* Gr]×(*ditto* Kostoff)

Evidence for the presence of sterility genes is also available from the partially fertile amphidiploids involving the several heterozygous races of *N. tomentosa*. These amphidiploids appear to differ in fertility, and certainly individual plants of the same race differ in this respect. The difference in fertility between amphidiploids from different races is due to the presence of more sterility genes in some than in others. That plants within a single line vary in fertility indicates that the race of *N. tomentosa* used

was heterozygous for the sterility genes. The difference in functional value between ovules of the same ovary indicates the heterozygous condition of the amphidiploid plant, some ovules receiving viable, others inviable gene combinations.

It was thought that if but a few chromosomes carried the genes responsible for female sterility, certain plants which had a different genic balance might be partially fertile. Crossing of autotetraploids would provide the opportunity for their occurrence.

That the fertility of the several amphidiploid lines is not due to chromosomal unbalance in the fertile plants but is a feature of the race of *N. tomentosa* used is shown by the following facts: In a study of the P.M.C. of 39115P18, a partially fertile plant, five cells each with 24 bivalents and two each with 23 bivalents+2 univalents were counted. In 39115P6, another partially fertile plant, four cells each with 24 bivalents and four cells each with 23 bivalents+2 univalents were counted. In addition, there is evidence from open and controlled pollinations of chromosomally unbalanced plants (recognized from their morphology) which shows that these are less fertile than the more nearly normal appearing plants. The most convincing evidence, however, is the high proportion of fertile plants in the lines 39113 and 39115 and the complete sterility of 54 plants of 39114 (table 3).

The action of the sterility genes is thought to be complementary. The genomes of the parental species of the sterile amphidiploids contain many similar or identical genes which affect the fundamental chemical-physiological processes common to both species. Embryo sac abortion is assumed to be due to the opposing reactions or rates of reaction of genes of the two species which influence the same phase of development—namely, embryo sac formation. As a result of this interaction, development ceases. There is reason to believe that a zygote once formed would be viable, for the amphidiploids themselves are exceedingly vigorous. If the hypothesis outlined above is correct, it should be possible to isolate fertile amphidiploids which are monosomic for those chromosomes carrying the complementary sterility genes, if the number of chromosomes is few.

METHODS

Pieces of ovaries were fixed, while evacuating, in Randolph's modification of Navashin's fluid (JOHANSEN 1940). Sections were stained with iodine-safranin-gentian violet (STOCKWELL'S 1934 modification of Flemming's triple stain). When evacuation was not feasible under field conditions, pieces of ovaries were first immersed for a few minutes in Carnoy's 3:1 before fixing with the above fluid. Differentiation was done with clove oil. A small amount of orange G added to the clove oil appears to improve

visibility of cell walls and cytoplasmic membranes. The preparations were mounted in Canada balsam. A few preparations of ovaries were stained by the Feulgen method, following fixation in acetic-sublimate (MARGOLENA 1932). The results were satisfactory, but a counterstain seems necessary to make it useful for general cytological work.

CYTOLOGICAL RESULTS

Megasporogenesis and Embryo Sac Development in Sterile Amphidiploids

The amphidiploids *N. sylvestris-tomentosa* and *N. sylvestris-tomentosiformis* Gr were obtained by the writer in the summer of 1937. Microsporogenesis in these amphidiploids is regular, and their pollen has been found to be 90 percent or more good. They are, nevertheless, completely female sterile. Some two hundred self- and cross-pollinations with *N. Tabacum*, *N. sylvestris*, *N. tomentosa*, and *N. tomentosiformis* failed on both amphidiploids. The same result also followed bud pollinations. No developing ovules could be detected in 48 ovaries examined with the binocular. Examination of styles dissected after heat treatment, stained in acid fuchsin, and mounted in lactic acid (BUCHHOLZ and BLAKESLEE 1922) revealed that the pollen tubes grow down the styles. These observations were later confirmed in styles embedded and sectioned in paraffin and stained with safranin-gentian violet. It then became clear that the difficulty lay in the ovules.

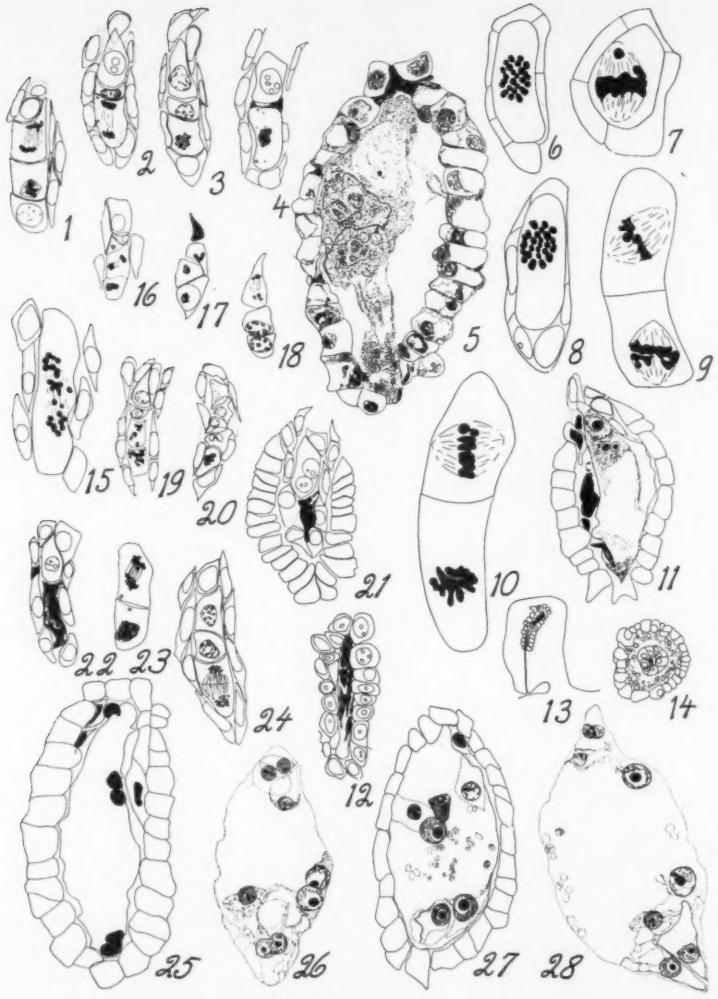
Buds of all sizes and flowers of both amphidiploids taken at frequent intervals up to seven and nine days after self-pollination were fixed (tables 1 and 2).

The cytological phenomena associated with the abortion of the ovules was studied in great detail in the amphidiploid *N. sylvestris-tomentosa* (Plate I, fig. 1–5; Plate II, fig. 1–6). Stages beginning with buds about three cm long (measured from the base of the ovary to the tip of the bud) and thence taken at short intervals up to nine days following pollination, were examined thoroughly. A similar, but less extensive, study was made of the amphidiploid *N. sylvestris-tomentosiformis* Gr (Plate I, fig. 6–14; Plate II, fig. 7–9). Megasporogenesis and embryo sac formation in the former will now be described.

In 2.6–2.9 cm buds, stages from the archesporial cell to the four megaspore condition were found. These stages were also found in 3.6 cm buds, but later stages may also be seen. The chromosome threads of prophase in the large archesporial cells may be made out clearly. Twenty-four pairs of chromosomes were counted in the IM polar view of three plates. Side views of IM plates also show normal behavior. Rather frequently, however, one unit or pair (?) of chromosomes may be seen lying off the plate,

but such variations do not affect the conclusion that meiosis is quite regular. The orientation of the IM spindle with respect to the longitudinal axis of the ovule is variable. Some lie parallel to it, others at an angle to it. As a result, the IIM plates may lie at different focal levels. The orientation of the IIM spindles may be at right angles to one another (Plate I, fig. 1), one oriented in the longitudinal axis of the ovule, the other at right angles to it, or both have been seen to lie at right angles to it. The result is that the four megaspores do not always lie in a straight line but take varying positions. The homeotypic division of the chalazal nucleus is often completed before that of the micropylar nucleus (Plate I, fig. 1–4). Indeed, the latter sometimes disintegrates while still in metaphase or anaphase (Plate I, fig. 3, 4). The chalazal megaspore is invariably seen to become the embryo sac mother cell (E.S.M.C.) (Plate I, fig. 1–4). This cell enlarges rapidly, while its sister cell soon disintegrates (Plate I, fig. 2–4). The walls separating the four megaspores apparently are not always formed. Only the wall separating the E.S.M.C. from its sister cell is usually present. As the embryo sac enlarges, disintegration of the nucellar cells may be noted, but some of these, toward the chalazal end of the sac, often persist for a long time. The narrow, long, deeply stained nuclei of these disintegrating cells which are wedged between the integument and the embryo sac are very typical.

In 4.7 and 5.6 cm buds and in later stages, one- and two-nucleate stages of embryo sac development are found. One-, two-, and four-nucleate stages are found in 6.6 cm buds when the flower is just beginning to open. At this time, also, some collapsed embryo sacs may be seen. Development does not proceed beyond the four-nucleate condition before degeneration begins (Plate I, fig. 5). At the comparable stage in *N. Tabacum*—that is, in recently opened flowers—two-, four-, and eight-nucleate sacs may be seen. Other fixations of the amphidiploid made 24, 32, 44, 48, 56, 64, 72, 96, 120, and 147 hours after pollination were studied. The latest fixations were made nine days following pollination. Throughout this period of time two- and four-nucleate embryo sacs (infrequently some nuclei of a sac are surrounded by membranes) were observed in various stages of disintegration. Remnants of nucellar cells are rare from about 24 hours on, but they may persist until 96 hours or so after pollination. The distribution of the nuclei or cells within the embryo sac is variable, but most often two are at the micropylar, and two at the chalazal end. After 96 hours they are often seen clumped together near the center of the sac, but still sharply defined. Some two- and, perhaps, four-nucleate embryo sacs are found even nine days after pollination, at which time almost all embryo sacs are collapsed. The space formerly occupied by the embryo sac is now filled by the huge integument cells which have encroached upon it. Sometimes,



EXPLANATION OF PLATE I

FIGURES 1–5.—Megasporogenesis and embryo sac development in *N. sylvestris-tomentosa* (4n). Figures 6–14. Meiosis and embryo sac development in *N. sylvestris-tomentosiformis* (4n) Gr. Figures 15–22. Megasporogenesis in F_1 *N. sylvestris*×*N. tomentosiformis*. Figures 23–28. Megasporogenesis and embryo sac development in F_1 *N. sylvestris-tomentosiformis* (4n) Kostoff× (*ditto*) Gr. All except figure 14 oriented with chalazal end upward. All except figures 7 and 14 longisections or median longisections. Figure 7 oblique-section. Figure 14 cross-section. Figures 6–10 and 13–15 cut at 15μ. Figure 12 cut at 10μ. All others cut at 20μ. Figures 1–5, 11, 16–23 ×500. Figures 6–9, 15×800. Figure 10×1133. Figure 12 ×400. Figures 13–14 ×90.

FIGURE 1.—Side view of homeotypic divisions showing precocious chalazal nucleus in telophase, micropylar nucleus in metaphase. 3.6 cm bud.

FIGURE 2.—Chalazal IIM completed. Functional megaspore greatly enlarged; its crescent-shaped sister cell starting to disintegrate. Micropylar nucleus in telophase. 3.6 cm bud.

Figure 3.—Chalazal IIM recently completed. Sister cell of functional megaspore (crescent-shaped cell) still prominent. Micropylar nucleus in metaphase; failure to complete IIM indicated. 3.6 cm bud.

FIGURE 4.—Chalazal IIM completed. Sister cell of functional megaspore disintegrated (dark strip across linear series). Chalazal nucleus disintegrating while still in IIM. 3.6 cm bud.

FIGURE. 5.—Two-nucleate embryo sac disintegrating nine days after pollination. Nucleoli still visible. Nuclear membrane dissolved. Numerous small vacuoles give nucleoplasm reticulate appearance. Nucellar cells absent.

FIGURE 6.—Megaspore mother cell in IM. Polar view showing about 24 bivalents. 3.2 cm bud.

FIGURE 7.—Megaspore mother cell in IM Side view. Note chromatin mass leading to pole. 3.2 cm bud.

FIGURE 8.—Megaspore mother cell in IM. Polar view showing probably 24 bivalents. 3.2 cm bud.

FIGURE 9.—Side view of IIM plates. The two divisions appear to be simultaneous and regular. 3.2 cm bud.

FIGURE 10.—Side view of chalazal IIM and polar view of micropylar IIM showing regular meiosis. 3.2 cm bud.

FIGURE 11.—Two-nucleate embryo sac 24 hours after pollination. Both nuclei toward chalazal end. Little evidence of nucellar cells. Integument cells normal. Disintegrated nucellar cell toward micropylar end (dotted angular area).

FIGURE 12.—Disintegrated embryo sac 24 hours after pollination. Dark regions probably indicate disintegrated nuclei. Prominent integument cells.

FIGURE 13.—Longisection of anatropous ovule 72 hours after pollination, showing micropylar canal leading to disintegrated embryo sac. Prominent integument cells.

FIGURE 14.—Cross-section of ovule 144 hours after pollination, showing overly developed integument cells surrounding disintegrated embryo sac. Prominent epidermal cells. Cells between integument and epiderm is also disintegrating.

FIGURE 15.—Side view of IM showing about 2 bivalents separating in equator of spindle. Single chromosomes lie scattered along the spindle. 4.9 cm bud.

FIGURE 16.—Probably five-celled condition. Wall formed around single unit. Small group of chromosomes dividing in one cell. 4.8–5.1 cm bud.

FIGURE 17.—Four-celled condition. Chalazal-most cell disintegrating. All contain chromosomes as left after IM. 4.8–5.1 cm bud.

FIGURE 18.—Three groups of chromosomes: two large, in interkinesis; one small, toward chalazal end in anaphase of IIM. Latter group of three chromosomes only. 4.8–5.1 cm bud.

FIGURE 19.—Four cells in a row. In two toward chalazal end chromosomes organized into nuclei. Micropylar-most in anaphase of IIM. Intermediate one with few chromosomes scattered in it. Flower open, anthers closed.

FIGURE 20.—Five cells in a row, each with chromatin. One cell with two nuclei. Micropylar-most appears to be in IIM. 4.8–5.1 cm bud

FIGURE 21.—Chalazal cell with two nuclei. Disintegrated cells toward micropyle. Nucellar cells enlarged. Integument cells prominent. Flower open, anthers closed.

FIGURE 22.—One chalazal cell. Several disintegrated cells toward micropyle. Enlarged nucellar cells. Flower open, anthers closed.

FIGURE 23.—Side view of IIM showing chalazal nucleus in anaphase; micropylar nulceus still in metaphase. Two chromosomes, one in each cell, in cytoplasm. 4.3 cm bud.

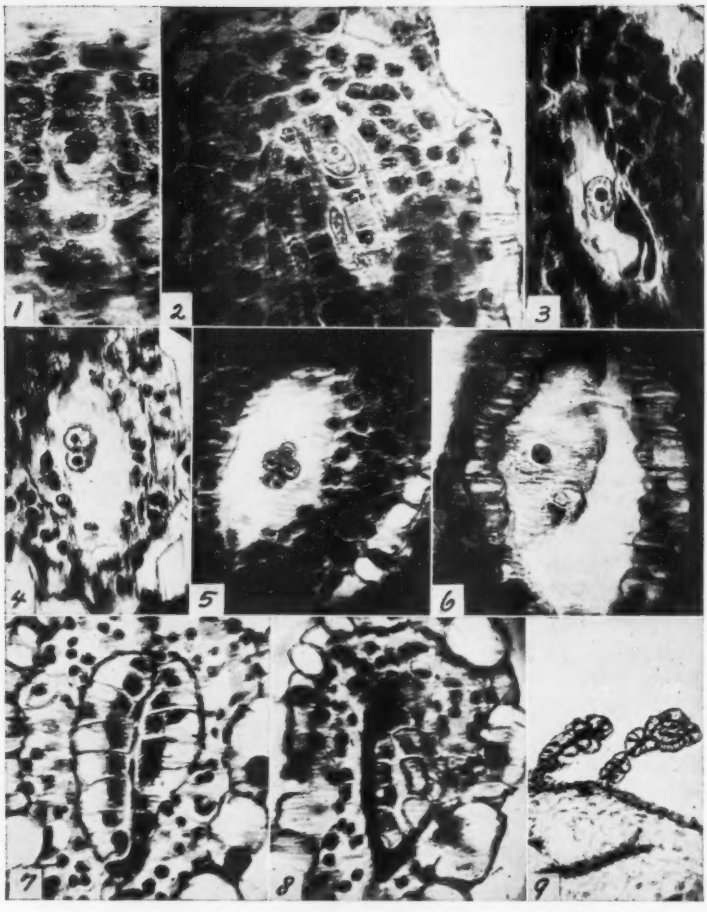
FIGURE 24.—Chalazal IIM completed. Cell plate just forming between micropylar chromosome groups. 4.3 cm bud.

FIGURE 25.—Four-nucleate embryo sac disintegrating 49 hours after pollination. A few disintegrating nucellar cells remain toward chalazal end.

FIGURE 26.—Eight-nucleate embryo sac 49 hours after pollination. Two large apposed polar nuclei to the right. Egg above and slightly to the left of two synergid nuclei. Antipodals at chalazal end. Note size relations of nuclei. From two serial sections.

FIGURE 27.—Four-nucleate embryo sac. The nucleus to left of large central nucleus unusually small. Disintegrated nucellar cell (stippled area) above large central nucleus. Another one at upper end and an enlarged one to the right below it. Note starch grains. From one serial section.

FIGURE 28.—Normally expanded eight-nucleate embryo sac 49 hours after pollination. Triangular synergids with egg above them. Polar nuclei approaching each other. Three small antipodal nuclei. Note starch grains. From two serial sections.



EXPLANATION OF PLATE II

Typical stages of megasporogenesis and embryo sac development in sterile amphidiploids. Figures 1–6 *N. sylvestris-tomentosa* (4n) and figures 7–9 *N. sylvestris-tomentosiformis* (4n). All ovules oriented with chalazal end upward. Figure 1 oblique-section; all other figures longisections or median longisections. Sections cut at 20 μ. Figures 1–3, 6×500. Figures 4, 5, 7, 8 ×300. Figure 9 ×60.

FIGURE 1. Polar view of megaspore motner cell in IM, showing 24 bivalents. 2.6–3.3 cm bud.

FIGURE 2. Chalazal IIM completed. Crescent-shaped sister cell of enlarged functional megaspore, with nucleus displaced to the left, disintegrating. Micropylar nucleus in telophase. Note intact nucellar cells surrounding linear quartet of megaspores. 3.6 cm bud.

FIGURE 3. Functional megaspore greatly expanded with the three disintegrated megaspores, or their equivalent, (dark masses) displaced toward side of sac. Nucellar cells disintegrating. Note expanding embryo sac accommodating itself to disintegrated megaspore mass. 3.6 cm bud

however, many of the integument cells have also disintegrated. Enlargement of integument cells from about 24 hours on is a very characteristic feature. This is not considered to be the cause for the disintegration of the female gametophyte, but rather a consequence of it.

The pollen tubes grow down the style. They have been seen in the ovarian cavity 72 hours after pollination (about the time when fertilization occurs in *N. Tabacum*). They have also been seen among the ovules at the base of the ovary in the other amphidiploid *N. sylvestris-tomentosiformis* Gr 96 hours after pollination. The generative nuclei within pollen tubes of the first amphidiploid have occasionally been seen. The shape of the generative nuclei varies from small deeply stained ovoid bodies to long wormlike structures. In the later fixations—that is, 96 hours or later—the nuclei in the pollen tubes appear to fragment, and degeneration occurs while many of the tubes are still in the style.

The amphidiploid *N. sylvestris-tomentosiformis* Gr shows very similar stages in its megasporogenesis and embryo sac development to those of the amphidiploid *N. sylvestris-tomentosa*. It differs from the latter in that the embryo sacs collapse earlier, so that almost none can be found for study 72 hours following pollination.

Since the behavior of *N. sylvestris-tomentosiformis* (4n) Gr corresponds very closely to that of *N. sylvestris-tomentosa* (4n), the former need not be described in detail. Drawings of meiosis and early stages of development are shown in Plate I, figures 6–11. Various later stages of development and disintegration are shown in Plate I, figures 12–14. The over-development of the integument is well shown, as is the resorption of cells lying between integument and epidermis. This resorption also occurs during normal testa development (SOUÈGES 1907). In the final stage only epidermis or integument plus epidermis remains. The reciprocal amphidiploid *N. tomentosiformis-sylvestris* behaves like the one just described, except that only two-nucleate stages of embryo sac development could be found throughout all later stages studied (table 1 and 2).

The amphidiploid *N. glutinosa-tomentosa* behaves exactly like the am-

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FIGURE 4. Two-nucleate embryo sac nine days after pollination. Nucellar cells absent.

FIGURE 5. Four-nucleate embryo sac 96 hours after pollination. Nucellar cells absent.

FIGURE 6. Same as Plate I, fig. 5.

FIGURE 7. Longisection of ovule 144 hours after pollination. Dark strips in center mark remnants of embryo sac. Note that resorption of tissue between prominent integument and epidermis has begun. Prominent epidermal cells.

FIGURE 8. Longisection of ovule 144 hours after pollination, showing resorption of cells between integument and epidermis (dark strip).

FIGURE 9. Final stage of ovule development nine days after pollination. Note that only epidermis and integument remain in the ovule to the right; only epidermis in the one to the left. Resorption complete.

phidiploid *N. sylvestris-tomentosa*. Meiosis and megasporogenesis in this amphidiploid is less regular, but no embryo sacs develop beyond the four-nucleate stage, and many disintegrate in the two-nucleate condition.

The amphidiploid *N. sylvestris-Setchellii* is similar in meiosis to the amphidiploid *N. glutinosa-tomentosa*. It occupies an intermediate position between the normal embryo sac development of *N. Tabacum*, where eight nuclei are present in the fully developed embryo sac, and the partial embryo sac development of the amphidiploids described above; for in it, two-, four-, and eight-nucleate embryo sacs are found in various stages of disintegration four days and later after self-pollination. This amphidiploid is almost or completely sterile. Although the majority of embryo sacs disintegrate before reaching the eight-nucleate condition, the complete sterility is not due to failure of embryo sac development alone. Examination of sections of styles, including the upper part of the ovary, has failed to show pollen tubes in the ovarian cavity four, five, six, eight, and nine days after self-pollination. Styles of flowers examined four and five days after pollination with *N. Tabacum* show only a few tubes near the base of the styles. The number of tubes gradually increases as one approaches the stigma end of the style. Pollen tubes have been seen in the tip of just one ovary three days after self-pollination. Ninety-three self-pollinations and fourteen pollinations with *N. Tabacum* have resulted in only one capsule with twenty "seeds," most of which apparently have no endosperm or embryo. Dissection has shown nothing more than diminutive, spherical masses of tissue containing, in some cases, very small embryos. Only two of the twenty "seeds" obtained would appear capable of germinating. Several bud pollinations were also tried with the same negative result. Plants left to open-pollinate in the field show a few developing ovules per pod, just as in the case of greenhouse material. This plant, nevertheless, gives some promise of being slightly fertile, perhaps when other pollen is used, or if the style is shortened, or by some other means.

Megasporogenesis and Embryo Sac Development in Sterile F_2 Segregates

Two sterile F_2 segregates from the F_1 *N. Tabacum*×*N. sylvestris-tomentosiformis* (4n) Gr, plant 11 and plant 95, were studied in detail (tables 1, 2). In plant 11 close attention was paid to the question of the coincidence of the two second metaphases and anaphases. The plant was found to behave like *N. Tabacum*—that is, the two divisions are usually simultaneous (*cf.* the four sterile amphidiploids). Development proceeds not further than the four-nucleate stage in this plant. One hundred and twenty hours after self-pollination many disintegrated ovules (table 2) were seen. The

nuclei of a sac can be recognized in only an occasional ovule at this stage. More than twenty self-pollinations on this plant failed. No developing ovules occurred.

No stages of meiosis were studied in plant 95, but in all later stages it was found to be very similar to plant 11 (table 1, 2). However, plant 95 shows an occasional apparently normally developing ovule (about 11 per pod as the maximum) but not enough to make the pod stay on the plant. Ten self-pollinations and ten pollinations with *N. Tabacum*, made under favorable conditions in the greenhouse, also failed, not a single pod remaining on the plant.

Megasporogenesis and Embryo Sac Development in Partially Fertile Hybrids

The F_1 hybrid *N. Tabacum*×*N. sylvestris-tomentosiformis* (4n) Gr also has quite regular megasporogenesis. The chalazal IIM is usually completed before the micropylar one, and therefore, in this feature, it resembles the four sterile amphidiploids already described. These amphidiploids usually show a precocious chalazal division. Stages from the fully open flower up to five days after self-pollination have shown two- and four-nucleate embryo sacs in various phases of disintegration. Healthy eight-nucleate embryo sacs were also found. The F_1 *N. Tabacum*×*N. sylvestris-tomentosa* (4n) very probably shows the same cytological behavior as the above one. Both hybrids, although appearing fertile and producing hundreds of good seeds per ovary, have only seventeen percent of functional ovules.

Very similar results to those just reported for the F_1 *N. Tabacum*×*N. sylvestris-tomentosiformis* (4n) Gr were also observed in the F_1 *N. Tabacum* ×*N. sylvestris-Setchellii* (4n). Again many ovules disintegrate in the two- and four-nucleate stages of embryo sac development, while others develop normally. This hybrid has only thirteen percent of functional ovules.

Megasporogenesis and embryo sac development in the F_1 *N. sylvestris-tomentosiformis* (4n) Kostoff×*ditto* Gr is of interest in connection with the sterility exhibited by the latter amphidiploid. Indeed, this seemingly fertile hybrid has only fourteen percent of good seeds. The manner of disintegration of the failing embryo sacs follows exactly that outlined for the sterile amphidiploids described above. Here also, the chalazal IIM is usually completed before the micropylar one (Plate I, fig. 23, 24). A disintegrating four-nucleate embryo sac of this hybrid is shown in Plate I, figure 25. A four-nucleate and two complete, normal eight-nucleate embryo sacs, showing the size relations of nuclei, may be seen in figures 27, 26, and 28, respectively, of Plate I. The eight-nucleate embryo sacs shown are also representative of normal embryo sacs in *N. Tabacum*. In *N. Ta-*

bacum, however, the two second meiotic divisions usually take place simultaneously. Just as in *N. Tabacum*, fusion of the polar nuclei does not occur until fertilization, and the antipodals persist at least until then. In all the plants studied the chalazal megaspore invariably was seen to give rise to the embryo sac.

Megasporogenesis in a Sterile F_1 Hybrid

It was thought desirable to compare meiosis and embryo sac development in the sterile F_1 hybrid *N. sylvestris*×*N. tomentosiformis* with that observed in plants having a balanced number of chromosomes. Also, since KOSTOFF employed this hybrid as the female parent in the synthesis of his fertile amphidiploid *N. sylvestris-tomentosiformis*, a study of megasporogenesis in this sterile F_1 hybrid should give some information concerning its possible fertility. Meiosis in this hybrid is highly irregular. From one to about three bivalents are formed at IM in most of the megasporocytes, while the remaining single chromosomes are scattered along the spindle. No metaphase plate is formed (Plate I, fig. 15). As a result, the chromosomes come to lie in groups made up of various numbers, all along the spindle. Each group of chromosomes, or even a single unit, then forms a wall isolating itself from the other groups (Plate I, fig. 16–20). Consequently, there are often more than two cells observed at the end of IM. Some of these may contain two nuclei (Plate I, fig. 20, 21). The group of chromosomes thus cut off, if sufficiently large, may, without forming a nucleus and without passing through interkinesis, immediately develop a second metaphase spindle, thus giving rise to additional cells (Plate I, fig. 18–20). The formation of supernumerary cells has its parallel in the formation of microcytes and micronuclei in microsporogenesis, the difference being that wall formation in the latter is delayed until after the end of IIM. As a result, there are probably fewer microcytes formed in microsporogenesis than in megasporogenesis. Figures 21 and 22 of Plate I illustrate a late stage in megasporogenesis. Only the chalazal cells remain, all others have disintegrated. Because the chalazal nucleus almost always fails to receive most of the chromosomes, disintegration soon takes place, no embryo sac being formed (*cf.* sterile amphidiploids). Very rarely an embryo sac develops. Only one was found in the sections studied, and it was atypical. It contained a large nucleus in the center of the sac and a small one directly adjoining it. A cell, simulating a synergid, could be seen at the micropylar end of the sac. The sac contained these three nuclei only. It appeared healthy and would seem to have been potentially functional. The observations reported explain the complete or almost complete sterility of this hybrid.

DISCUSSION

A search through the literature of the past fifteen years failed to reveal any cases of amphidiploids which, like *N. sylvestris-tomentosiformis* (4n) Gr and *N. sylvestris-tomentosa* (4n), exhibit genic sterility. The proof that the sterility is genic is given by the regular pairing of the chromosomes at meiosis. The sterility is unique, for all the female gametophytes are eliminated, but the pollen is not affected. This indicates that the sterility in the corresponding F_1 hybrids is genic as well as due to chromosomal unbalance of the gametes.

A number of partially fertile amphidiploids are reported by POOLE (1932), but in all of these sterility is presumably due to chromosomal unbalance in the gametes following irregularities in chromosome pairing at meiosis. Therefore, both the male and female gametes are affected. These cases are very different from those herein reported. The cause of sterility in the sterile amphidiploids is physiological and is probably due to the action of complementary sterility genes in an otherwise balanced and stable genetic system.

Megasporogenesis and embryo sac development in sterile and partially fertile Nicotiana species hybrids have been studied by BRIEGER (1928a, 1928b). BRIEGER also observed some of the phenomena reported in this paper—for example, precocious completion of IIM and arrested development of embryo sacs in the two-nucleate, four-nucleate, or later stages. No explanation was given other than that chromosome unbalance led to abortion of the gametes.

Failure of normal embryo sac development has been reported for a number of species belonging to different families, or even orders, of the plant kingdom, but in all of these adequate genetic knowledge of the material is lacking, and the cause of the abortion remains obscure. Also, in these cases there is always some seed formed—that is, some of the ovules, for no obvious reason, develop while others disintegrate. Such a situation has been reported by SOUÈGES (1926) for *Papaver Rhoeas*, another by MAHESHWARI (1931) for *Albizzia lebbeck*, a member of the Mimosaceae, and still others by various authors. Disintegration in *Papaver Rhoeas* seems to be due to lack of proper organization of the nuclei in the fully developed embryo sac. In *Albizzia lebbeck* disintegration may occur at any stage from prophase of the archesporial cell to the fully developed embryo sac. A similar case has been reported by LANDES (1939) for rye, but here the cause is probably chromosomal, for it is accompanied by a corresponding amount of pollen abortion.

All the cases of embryo sac abortion except one (SINGLETON and MANGELSDORF 1940) which the writer has encountered in the literature are dif-

ferent from those in the sterile amphidiploids, and all that is learned from them is the cytology of abortion.

Instances which resemble the sterility in the amphidiploids are the genes causing male sterility in *Zea mays* (BEADLE 1932). These affect pollen formation only, for the plants are female fertile. All are simple Mendelian recessives. The different ones cause a breakdown in gametogenesis anywhere from prophase to the first division in the pollen grain. The cases which particularly resemble the sterile amphidiploids are those which cause abortion between the end of meiosis and the first division in the pollen grain. Cases similar to the male steriles of maize have been reported for Hebe by FRANKEL (1940) and for Viola by CLAUSEN (1930). The cause of abortion in these plants is unknown, but it is thought to be genic in the former, chromosomal in the latter.

The only case of a female gametic lethal, and the one which most nearly resembles the action of the sterility genes in the sterile amphidiploids, has been reported by SINGLETON and MANGELSDORF (1940). This is the *lo* (lethal ovule) gene in maize. This gene, which is closely linked with *su* (sugary), causes abortion of the ovules which receive it, thus distorting the ratios of starchy to sugary. The gene is maintained only in the heterozygous condition by transmission through the pollen. No cytological study has been reported of this case in maize.

Another case which resembles the sterile amphidiploids is that of male sterility in the interracial hybrids of *Drosophila pseudoobscura* (DOBZHANSKY 1936). This investigator has shown that sterility genes in the races A and B of this species are scattered through the genetic system. They have been found in each of the four largest chromosomes tested and have been demonstrated for both arms in the X and in the II chromosome.

The occurrence of sterility genes is of significance in the problem of the origin of isolation mechanisms between forms, thus making possible the differentiation of species. To the example of intraspecific isolation in Drosophila can be added a form of interspecific isolation shown by one race of *N. tomentosa* and by *N. tomentosiformis*. Those forms of the *N. tomentosa* group which give sterile amphidiploids may represent inadvertent selections for sterility genes in the originally heterozygous species, just as there appear to be differences in fertility between amphidiploids of the same heterozygous, partially fertile lines today. The existence of these sterility genes in this case has not led to isolation between races of *N. tomentosa*, for they are all apparently interfertile.

A possible case in which a racial difference may have given a fertile amphidiploid is that of *N. glutinosa-tomentosa* (4n). ELVERS (1934) mentioned that a spontaneous amphidiploid of these two species which occurred in an F_1 population of *N. glutinosa* (U.C.B.G. 31-21) × *N. tomentosa*

(U.C.B.G. 08-193) was fertile. No description of the fertile amphidiploid has apparently ever been given, and the statement by ELVERS regarding its fertility may have been based solely on the good pollen produced by this amphidiploid which may actually have been female sterile like the one made by the writer involving *N. glutinosa* (U.C.B.G. 07-79) and *N. tomentosa* (U.C.B.G. 08-193).

Of all the amphidiploids made, *N. sylvestris-tomentosiformis* Gr perhaps resembles the majority of the cultivated varieties of *N. Tabacum* most closely in vegetative features and in the form of the flower. It is not possible, however, on the basis of morphology alone to decide which of the races or species of the *N. tomentosa* group is most like the original progenitor, for, since its origin, *N. Tabacum* itself has become changed under cultivation by mutation and selection.

In the light of the present knowledge *N. Tabacum* most probably originated as an amphidiploid between *N. sylvestris* and a form of *N. tomentosa* similar to one of the several races which today give partially fertile amphidiploids. Following its origin, the parental species of *N. tomentosa* may have given rise to the several races of *N. tomentosa* which exist today. Selection by inbreeding within certain races may then have isolated those homozygous types which today give sterile amphidiploids. It is probable that if a number of amphidiploids were made by the callus method from F_1 hybrids between *N. sylvestris* and any one of the several heterozygous races of *N. tomentosa* from the wild, there would be some fertile and some sterile ones among them. Selection within such races should produce varieties giving either sterile or fertile amphidiploids. On the other hand, it is quite possible that many races of *N. tomentosa* already existed at the time *N. Tabacum* originated. In any case it should be possible to isolate a fully fertile amphidiploid from the partially fertile amphidiploids. If it should be found that some of the heterozygous races of *N. tomentosa* from the wild give fully fertile amphidiploids, while others give sterile ones, the distribution of the races giving fertile ones should indicate the region of origin of *N. Tabacum*. From the available evidence this region is probably in Peru.

The sterility of some racial combinations does not necessarily eliminate them as ancestors, for, if *N. Tabacum* arose from unreduced gametes of the sterile F_1 hybrid or by a method similar to that employed by KOSTOFF, certain combinations carrying sterility genes would be eliminated as gametes. If, as is also possible, *N. Tabacum* originated by zygotic doubling or as a bud sport of the F_1 hybrid, perhaps under conditions of cultivation (*cf. N. digluta* and *Primula kewensis*), a race of *N. tomentosa* would be needed which did not carry the sterility genes.

Little has been said of *N. sylvestris*. Similar intraspecific differentiation has occurred within this species. Two races are known to the writer, but

more may exist. Only one has given vigorous hybrids. The other (U.C.B.G. 37-11), when crossed with *N. tomentosiformis* and with *N. Setchellii*, has given dwarf hybrids which grow very abnormally and retain only an apical tuft of leaves. These hybrids have not flowered. Profound physiological disturbances evidently are the cause for their abnormal growth. For this reason no amphidiploids have been made using this race.

The present problem is one in evolution. The ultimate goal of this phase of the tobacco investigations is to determine how evolution in *N. Tabacum* has occurred since its origin as an amphidiploid from the two parental species. Herein lies the value of these *Tabacum*-like amphidiploids which make it possible to determine in which genes they differ from *N. Tabacum*. They also aid in discovering which genes of *N. Tabacum* are present in duplicate and which duplications, now existing in the sterile amphidiploids, have been eliminated in *N. Tabacum*. Finally, they permit new genes to be introduced into *N. Tabacum* for further investigations.

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SUMMARY

Four amphidiploids—*N. sylvestris-tomentosa*, *N. sylvestris-tomentosiformis*, *N. sylvestris-Setchellii*, and *N. glutinosa-tomentosa*—were made by the callus method.

The amphidiploids *N. sylvestris-tomentosa* and *N. sylvestris-tomentosiformis*, in spite of having regular meiosis in megasporogenesis as well as in microsporogenesis and having over 90 percent of good pollen, are completely female sterile. This proves that the sterility is genic and that the sterility in the corresponding F_1 hybrids is genic as well as chromosomal. The other amphidiploids *N. sylvestris-Setchellii* and *N. glutinosa-tomentosa* which have somewhat less regular meiosis are also female sterile.

Examination of ovaries of the sterile amphidiploids *N. sylvestris-tomentosa*, *N. sylvestris-tomentosiformis*, and *N. glutinosa-tomentosa* revealed that megasporogenesis is quite regular, but that embryo sac development does not proceed beyond the two- or four-nucleate stage when disintegration occurs. Only the amphidiploid *N. sylvestris-Setchellii* showed some eight-nucleate embryo sacs.

A working hypothesis of the cause of the sterility in the amphidiploids was evolved. Only those megaspores which do not carry the sterility genes can give rise to functional embryo sacs. These genes are invariably present in the megaspores of the sterile amphidiploids. They do not, however, affect the viability of the pollen.

Three different, partially fertile amphidiploids were made by crossing a callus-induced autotetraploid of *N. sylvestris* with three, similarly induced heterozygous autotetraploid races of *N. tomentosa*. The major sterility genes, at least, are considered to be absent in the fertile ovules of the partially fertile amphidiploids.

It is concluded that *N. Tabacum* arose as an amphidiploid from *N. sylvestris* and some one of the several races of *N. tomentosa* which today give partially fertile amphidiploids with *N. sylvestris*. The probable place of origin is that region in central South America (Peru, Bolivia, and northwest Argentina) in which these plants occur today.

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A RECONSIDERATION OF THE MODE OF DEVELOPMENT OF THE BAR EYE OF DROSOPHILA MELANOGASTER[1]

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INTRODUCTION

THE sex-linked semi-dominant mutation, Bar, of *Drosophila melanogaster* (TICE 1914) when homozygous reduces the facet number of the compound eye from approximately 770 (the facet number of the wild type) to about 75. When heterozygous, it reduces the facet number to about 350. Its discovery afforded geneticists an effective tool for the quantitative study of gene action. Only one year after the announcement of its discovery, ZELENEY and MATTOON (1915) showed it to be sensitive to selection—that is, to changes in the genotypic environment. SEYSTER (1919) showed that in Bar facet number varied inversely with temperature and that temperature was effective only during the larval period. To explain his data, SEYSTER postulated "the existence of a chemical factor or determiner which acts as an inhibitor of facet formation and that, at a higher temperature, the speed of reaction is much greater than at a lower." This pioneer work of SEYSTER, which unfortunately was interrupted by the first World War, opened the floodgates for a number of experiments designed to measure the effect of temperature on the development of the Bar eye, with the intention of finding an explanation of the mode of action of the Bar mutant.

KRAFKA, in a series of three papers, published in 1920, confirmed and extended SEYSTER's work to show that temperature was effective during only a limited portion of the larval period. He suggested " . . . the decrease in facet number to be due to an inhibitor, the temperature coefficient of which differs from that of the normal facet-producing reaction . . ." and that " . . . the Bar eye factor comes into play after about three-fourths of the larval period is finished." Subsequent work showed that the temperature effective period (T.E.P.) varies in duration (percentage of larval life) and position from temperature to temperature (DRIVER 1926 and others). For example, at 20°C the T.E.P. occupies approximately 25 percent of larval life, while at 27°C it includes only about 15 percent (DRIVER 1931).

More recently, MARGOLIS (1934, 1935a, 1935b, 1936) very carefully repeated the temperature work on Bar. His conclusions in the main confirm those of the earlier workers. MARGOLIS (1935a) presented in schematic

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form a hypothetical system of reactions to explain the action of the Bar mutant. This scheme was based largely on the conclusions of KRAFKA as well as on MARGOLIS's own data. It postulates a substance or substances " . . . whose quantity at a particular time determines the number of facets," the so-called "facet precursor," and that Bar initiates (MARGOLIS 1935a) or accelerates (MARGOLIS and ROBERTSON 1937) a chain of reactions resulting in a product which combines with the "facet precursor," thus reducing its quantity and consequently the facet number. The interaction of the two "products," the "facet precursor" and the "Bar substance" occurs during the T.E.P. and is limited by the extent of that period. The limits of the T.E.P. are determined by two factors: (a) its onset is determined by the formation of the products resulting from the chain of reactions initiated or accelerated by Bar (CHEN (1929) observed that at the early stages of larval development the eye discs of Bar and wild type larvae are not different, while at later stages they are markedly so); (b) its end depends upon the "great many. . . . processes of general development which determine when differentiation of the optic discs into ommatidia may take place" (MARGOLIS 1935a). KRAFKA (1924) placed this time at three to four days after oviposition.

HUXLEY (1935) offered an alternative hypothesis to that of KRAFKA and MARGOLIS. He claimed that the data on Bar indicate " . . . that the main difference between Bar and full eye is brought about by differential growth during late larval life." HUXLEY's hypothesis led him to draw the same conclusions concerning the growth curve of Bar eye discs as did GOLDSCHMIDT (see below) with the one difference that HUXLEY recognized the possibility that Bar might reduce the size of the eye disc before as well as during the T.E.P. The main reduction, through the alteration of the growth rate, would occur, however, during the T.E.P.

MEDVEDEV (1935) compared the growth curves of glass^2, eyeless^2, Lobe^e, and wild type. He found that the eye discs of the mutants were smaller than those of wild type at 36 hours of larval life. (He started his measurements at 24 hours of larval life, but due to the small size of the cephalic complex at this time, it seems to the present author that these data are unreliable.) He found further that all four types had the same growth rate for the remainder of their development.

The observations on the T.E.P. coupled with those of KRAFKA and CHEN on the morphology of the eye disc lead GOLDSCHMIDT (1938) to believe that a comparison of the growth curves of Bar and wild type eye discs would yield quite different results from those derived by MEDVEDEV. GOLDSCHMIDT, in discussing the Bar case (1938, page 31), says " . . . certain experimental facts point to the possibility that in this case a phenomenon may occur similar to that observed in the vestigial case, viz.,

a secondary though rather early destruction of already formed eye material." The expectation in the case of Bar, then, would be that with the onset of the T.E.P. the Bar eye discs would become relatively smaller than the wild type, although both would continue to increase in size. If the conclusions discussed above concerning the growth curve of Bar eye discs are true, we would have rather forceful evidence that the definitive effect of Bar on facet number occurs during the T.E.P.—that is, during a limited and well defined period of larval development. It should then be possible to proceed from this point to study by other means—for example, chemical and histological—the exact nature of the Bar reaction.

Clearly then, the answer to the question "When does the definitive effect of Bar on facet number occur?" would be extremely useful in seeking an answer to the larger question "How does Bar affect facet number?" Each of these questions actually includes a series of smaller questions as follows:

(1) When does the effect of Bar on facet number occur? Is it before, during or after the T.E.P., etc.?

(2) How does Bar limit facet number? Is it by an effect on growth rate as postulated by HUXLEY and GOLDSCHMIDT; or by growth limitation as SINNOTT and his students have found for fruit size in various Curcurbits (see SINNOTT and DUNN (1935) for review); or by resorption of already formed material as in Brachyury and Taillessness in the house mouse (CHESLEY 1935, GLUECKSOHN-SCHOENHEIMER 1938); or by destruction (cytolysis) of already formed material as GOLDSCHMIDT postulates for the vestigial case in *Drosophila melanogaster* (GOLDSCHMIDT 1937); or does Bar affect facet number simply by reducing the initial capital of cells, etc.?

The method employed to seek answers to these questions consisted primarily in a comparison of the growth curves of the eye discs of Bar and wild type. This method involves the assumption that there is a direct and consistent relation between the size of the eye disc in the mature larvae and the size of the eye of the imago as measured by facet number. That such an assumption is valid is adequately shown by the observations of such correlation for the mutants glass^2, eyeless^2, Lobe^c (MEDVEDEV 1935), Bar (CHEN 1929), and the Bar "alleles" (STEINBERG and ABRAMOWITZ 1938) as well as for the wild type.

Preliminary reports of various aspects of this work were given in DROSOPHILA INFORMATION SERVICE 11, at the Seventh International Congress of Genetics and at the Genetics Society meetings in Woods Hole, August 1940.

MATERIALS AND METHODS

The growth curves of the eye discs were determined by the technique originally described by MEDVEDEV (1935). The method consists of making

camera lucida drawings of the living eye discs immediately after they are dissected in a drop of Ringer's solution from larvae of known ages. Planimeter measurements of the areas of these drawings are then made. Because the discs are slightly curved it is necessary to flatten them by gentle tapping with a needle. Variation in the flattening, of course, is a source of error in the measurements. Other sources of error are the inaccuracies introduced in the camera lucida drawings and in the measurements of these drawings. These errors, however, are random and tend to cancel out. This is shown by the fact that the measurements can be reproduced.

The ages of the larvae were determined as follows: eggs were collected over a 24 hour period at $25 \pm 1°C$ by the method described by SCHWEITZER (1935). Newly hatched larvae were collected over a two hour period. Consequently all ages recorded in the paper are from hatching and are accurate to within $\pm$one hour. The larvae were raised at $27 \pm 1°C$ (unless otherwise indicated) in three inch Petri dishes containing 15 to 20 cc of the following food: 2 percent agar, plus 12.5 percent of molasses in H_2O, seeded 24 hours before use with two drops of a heavy yeast suspension. Thirty larvae were placed in a dish.

Measurements of the eye discs were made at 12 hour intervals from 36 hours after hatching until 84 hours after hatching at which time approximately one-half of the larvae had pupated.

Eye discs (cephalic complex) of 24 hour old larvae were not measured, because the magnitude of the error of measurement introduced by the minute size of the discs at this time makes such measurements meaningless. In addition, even at a magnification of 100× it is not possible to be certain that it is the cephalic complex and not some other tissue that is being measured.

The following stocks of *Drosophila melanogaster* were employed: (1) A Florida wild type strain, which had been inbred for more than 100 generations; (2) an inbred Bar strain which had been rendered isogenic with the wild type by repeated back-crossing and which, by the beginning of this experiment, had been inbred for approximately 100 generations.

No measurements of the cephalic ganglia of the larvae were made, because preliminary examination indicated that Bar probably did not affect the size of the cephalic ganglia. This agrees with MEDVEDEV's observations that the mutants eyeless^2, Lobe^e, and glass^2 do not affect the size of the cephalic ganglion even though they have a marked effect on facet number and optic disc size. The observations that glass^2 (JOHANNSEN 1924), eyeless^2 (RICHARDS and FURROW 1925, DERRICK 1928), and Bar (KRAFKA 1924) reduce the optic tract in the adult brain indicate that an investigation of the nature of the dependence of the adult brain on the eye would be fruitful.

Other materials and techniques used will be described below.

DATA

In view of the fact that both CHEN (1929) and MEDVEDEV (1935) offer extensive descriptions of the cephalic complex and of the eye discs of *Drosophila melanogaster* and that these discs closely resemble the discs of Melaphagus described by WEISMANN (1864), such description will be omitted here. CHEN offers excellent drawings of both Bar and wild type eye discs. The present data entirely confirm MEDVEDEV's description of the course of development of the wild type eye disc with respect to its gross morphology. As in MEDVEDEV's work, it was found that the cephalic complex does not separate into eye and antennal discs until some time between 36 and 48 hours after hatching. MEDVEDEV, in discussing the mutant eye discs, states that the "Morphological resemblance of the imaginal disc and the adult eye consists in the very early appearance of the asymmetry of the former, beginning from the separation of discs from the cephalic complex." It will be recalled that MEDVEDEV worked with ey^2 and L^e, both of which are very asymmetrical phenotypically. Bar, on the other hand, is quite symmetrical; in fact, it is identical in shape, but not in size, with the wild type eye disc (CHEN 1929 and the present work). A discussion of the histological structure of the eye disc will be reserved for a later section of the paper.

Tables 1 and 2 present the data obtained from the measurements of the various eye discs. The data show clearly that growth is rapid at first and then gradually falls off. This is further demonstrated in figure 1 and table 3. Figure 1 is based on the data of table 2, while table 3 is calculated from the data in table 2. This change in growth rate had been observed previously by MEDVEDEV and also by ENZMANN and HASKINS (1938) for the eye discs and the dorsal ganglia and is, of course, what has been observed repeatedly in all measurements of this type.

Comparison of the growth curves of Bar and wild type eye discs

The Bar eye disc, or rather the Bar cephalic complex, is clearly and significantly smaller than that of the wild type at 36 hours of larval life. The mean area of the camera lucida drawings of the Bar eye discs equals 151.4 ± 6.3 sq. mm, while that of the wild type equals 177.8 ± 4.8 sq. mm (table 2 and fig. 1). The difference in size between the Bar and wild type eye discs is equally clear at 48 hours (Bar $= 345.3 \pm 6.7$, wild type $= 543.6 \pm 9.1$). At this age the optic discs may be directly compared with each other, since at this time the cephalic complex has separated into the eye and antennal discs. Reference to tables 1 and 2 will show that the differences in size are equally marked at 60, 72, and 84 hours of larval life.

TABLE 1

Areas in square millimeters of the camera lucida drawings of +, B and B;m(B) eye discs taken from larvae at the ages indicated in the table. Magnification = 140×. Temperature = 27 ± 1°C.

GENOTYPE	♀♀			♂♂		
	MEAN ± σ_M	C_v	N	MEAN ± σ_M	C_v	N
			36 hours			
+	182.8± 5.8	22.0	47	171.1± 8.0	27.6	35
B	154.5± 6.3	22.4	40	153.6± 4.9	19.6	37
B;m(B)	133.3± 3.8	20.9	54	143.0± 5.9	21.5	27
			48 hours			
+	572.9±11.7	15.3	57	489.7±14.4	16.5	31
B	362.0± 6.8	10.7	33	332.7± 7.5	14.9	44
B;m(B)	336.6±14.2	24.2	33	340.9± 6.0	12.2	48
			60 hours			
+	946.5±19.8	14.8	52	938.5±15.5	9.4	33
B	773.7± 9.8	9.5	56	720.9±16.5	11.0	23
B;m(B)	629.9±10.6	11.0	42	642.4±12.2	11.0	34
			72 hours			
+	1138.9±25.9	14.3	40	1127.8±40.9	20.7	32
B	914.9±11.6	8.6	46	803.5±15.1	10.9	34
B;m(B)	854.0±12.5	9.4	41	836.9±13.8	10.2	39
			84 hours*			
+	1347.0±42.4	19.5	38	1345.6±32.5	15.0	39
B	1005.8±20.7	11.3	30	†895.0±27.3	17.7	34
B;m(B)	1036.4±13.1	8.4	45	1023.2±20.0	11.1	33

* At this time approximately one-half the larvae had pupated. Only those larvae which had not pupated were used; consequently these values may be lower than what would have been obtained from a random sample.

† This value appears to be too low as evidenced by comparison with the females and also by comparison with the values in table 5. See the text for further discussion.

Relation to the T.E.P.

DRIVER (1926) has shown that at 27°C the T.E.P. begins at about the 60th hour of egg-larval life and ends 15 hours later—that is, after 75 hours of egg-larval life are completed. According to POWSNER (1935) the length of the egg stage at 27°C in the wild type line with which he worked is 13 hours. If it is assumed that this is true, or approximately true for the Bar stock used in these experiments, the T.E.P. at 27°C begins at sometime between 45 and 50 hours of larval life and ends after approximately 60 to 65 hours of larval life are completed. It is clear, therefore, that the measurements which were made at 36 hours after hatching were made well before the T.E.P. began, while those made at 48 hours of larval life were made at approximately the time of onset of the T.E.P. It will be recalled that

TABLE 2

Same as table 1 except that the values given here are derived from the combined male and female data.

GENOTYPE	MEAN $\pm \sigma_M$	C_v	N
	36 hours		
+	177.8± 4.8	24.3	82
B	154.1± 4.0	22.8	77
B;m(B)	136.5± 3.2	21.1	81
	48 hours		
+	543.6± 9.1	15.7	88
B	345.3± 6.7	17.1	77
B;m(B)	337.9± 6.8	18.1	81
	60 hours		
+	943.4±13.3	13.0	85
B	758.3± 7.3	8.6	79
B;m(B)	635.5± 8.0	11.0	76
	72 hours		
+	1134.0±23.1	17.3	72
B	867.6± 9.2	9.4	80
B;m(B)	845.7± 9.3	9.8	80
	84 hours		
+	1346.3±26.5	17.3	77
B	946.9±17.9	15.1	64
B;m(B)	1038.8±11.3	9.6	78

TABLE 3

Growth increments of +, B and B;m(B) eye discs. (Calculated from the data of table 2.)

GENOTYPE	48/36	60/48	72/60	84/72	AVERAGE
+	3.06±0.09	1.74±0.04	1.20±0.02	1.19±0.03	1.80±0.26
B	2.24±0.07	2.19±0.05	1.14±0.02	1.09±0.02	1.67±0.20
B;m(B)	28.4±0.08	1.88±0.04	1.33±0.02	1.23±0.02	1.73±0.23

according to the hypothesis as expressed by GOLDSCHMIDT (1938)—that is, "a secondary . . . destruction of already formed eye material"—the eye discs of Bar and wild type may be expected to be the same size before the T.E.P. and to become different only after the T.E.P. has begun. This change in relationship is accredited to the presence of different growth rates in the two forms during the T.E.P. The data show conclusively that the Bar eye discs are smaller than the wild type at 36 hours of larval life—that is, well before the T.E.P.

HUXLEY (1935), as stated above, did recognize the possibility that Bar might reduce the size of the cephalic complex (eye disc) before the T.E.P.,

although he, like GOLDSCHMIDT, postulated that the main effect of Bar would be detected as a reduction of the growth rate during the T.E.P. A comparison of the Bar and wild type eye discs at the beginning and end of the T.E.P. should afford a simple and direct test of this assumption. If the Bar eye discs have a slower rate of growth during the T.E.P. than do those of the wild type, the ratio of the area of the Bar discs at the end of the T.E.P. to that at the beginning should be less than the same ratio

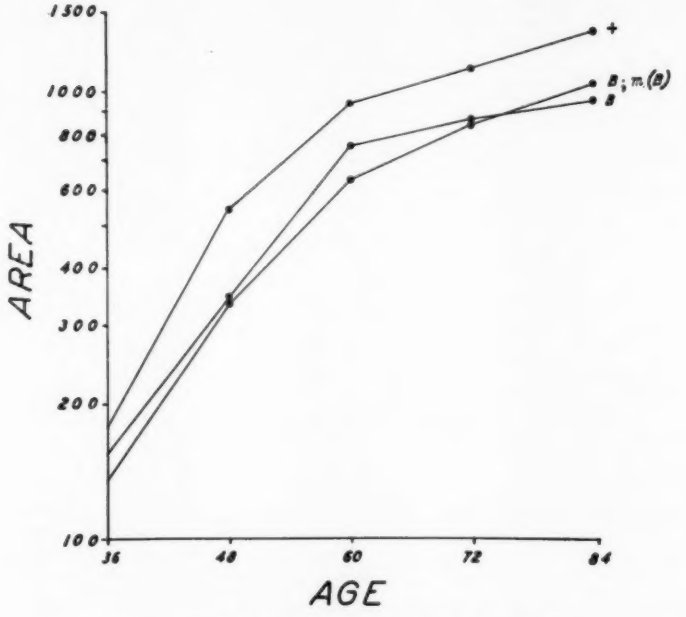


FIGURE 1.—Semi-logarithmic plot of the data in table 2.

for the wild type. It has been shown above that probably all of the T.E.P. at 27°C falls between the 45th and 65th hours of larval life. Consequently a comparison of the ratios of Bar at 60 to Bar at 48 hours with that of wild type at 60 to wild type at 48 hours is almost exactly what is required. The column headed 60/48 of table 3 contains the necessary data. The ratio for Bar is 2.19 ± 0.05; that for wild type is 1.74 ± 0.04. The growth rate of Bar, contrary to what would have been predicted from the hypothesis, is higher rather than lower than that of wild type. (It will be shown below that these values are probably the same.)

The growth rates of Bar and wild type between 60 and 72 hours and

between 72 and 84 hours are statistically the same, the corresponding values being 1.14 and 1.09 for Bar and 1.20 and 1.19 for wild type (table 3). It is clear, therefore, that there is no reduction in the growth rate of the Bar eye discs relative to that of the wild type eye discs either during or after the T.E.P. It can be shown by elementary algebra that the apparent greater growth rate of wild type as compared to that of Bar during the 36 to 48 hour interval is due in large part and perhaps entirely to the fact that at 36 hours the cephalic complex has not separated into optic and antennal discs while at 48 hours it has (the antennal discs of Bar and wild type are the same). Since the actual size of the antennal disc at 36 hours cannot be determined, no direct comparison of the growth rates of Bar and wild type optic discs can be made for the 36 to 48 hour interval.

The above data indicate that the growth curve of the Bar eye discs bears the same relationship to that of the wild type as do those of glass[2], eyeless[2], and Lobe[e]. Consequently, neither of the hypotheses mentioned above explains the data and both must therefore be discarded. A more extensive discussion of these data will be presented below.

Comparison of the growth curves of Bar and modified Bar eye discs

The mutant *m(B)* is a semi-dominant modifier of Bar. It was found originally in two *B;px sp* males with exceptionally large eyes and has been maintained in a stock having this genotype. Subsequent work has shown the modifier to be located in the second chromosome approximately six units to the left of *px*. It has also been shown that *m(B)* does not have an effect on facet number in the absence of Bar or one of its 'alleles' (STEINBERG in press). For the purposes of this paper *px* (plexus) and *sp* (speck) may be ignored (STEINBERG in press). This stock was inbred but had not been backcrossed to either the Bar or wild type stocks.

At 25°C modified Bar males have 220 facets; the females have 141 facets. In the Bar stock used in these experiments the males have 74.2 facets and the females 75.2 facets at 25°C. At 29°C the facet number of the modified Bar males is 169.7 and that of the females 114.5; those of Bar are 38.0 and 35.7 in the males and females respectively (STEINBERG in press). It is apparent that an increase of temperature has less effect on facet number in the case of modified Bar than in the case of Bar. No counts of facet number were made at 27°C. Nevertheless, it may be concluded that the facet numbers of Bar and modified Bar at this temperature are intermediate between those at 25° and 29°C. Equally, the difference in facet number between Bar and its modified form will be intermediate to that at 25° and 29°C. Therefore, it was expected that the eye discs of modified

Bar would be larger than those of Bar, since previous work had shown a correlation between facet number and disc size. Tables 1, 2, 3, and 4, and figure 1 present the pertinent data.

Considering the sexes at each age separately (table 1), the females show the following relationships: at 36 hours there is a barely significant difference (Diff. $= 21.2 \pm 7.3$) which is in the opposite direction to that expected ($B = 154.5 \pm 6.3$, and $B;m(B) = 133.3 \pm 3.8$); at 48 hours the discs are the same size statistically (Diff. $= 28.4 \pm 15.7$); at 60 and 72 hours the discs are significantly different in size, but contrary to expectations, the Bar discs are larger than the modified Bar ($B = 773.7 \pm 9.8$ and 914.9 ± 11.6, and $B;m(B) = 629.9 \pm 10.6$ and 854.0 ± 12.5); at 84 hours the discs are again statistically the same (Diff. $= 30.6 \pm 24.5$); the males on the other hand show significant differences in size at 60 and 84 hours of age only. At 60 hours the Bar eye discs are larger ($B = 720.9 \pm 16.5$, and $B;m(B) = 642.4 \pm 12.2$), while at 84 hours the modified Bar eye discs are larger ($B = 895.0 \pm 27.3$, and $B;m(B) = 1023.2 \pm 20.0$). Because of subsequent measurements made on Bar eye discs from mature larvae, it is believed that there was some undetected experimental error involved in the measurement of the male Bar eye discs at 84 hours. These measurements were made to test a hypothesis advanced in a later section of this paper. They will be discussed in that light below. For the present, it will suffice merely to indicate that measurements were made of the eye discs of mature Bar larvae which had completed their development at either 20°, 25° or 28°C, after spending the first 24 hours of their larval development at 25°C. All the larvae were therefore of the same physiological age. The data are presented in table 5. The magnification here was 146 diameters as compared with 140 diameters in the earlier measurements. The correction factor for translating the values of table 1 into those of table 5 is therefore $(146)^2/(140)^2$, or 1.09. This gives a corrected value of 1096.3 for the 84 hour old Bar females and 975.6 for the corresponding males. The corrected value for the females agrees very well with the values of table 5, while in the case of the males it is clearly much lower. Since all three of the mean areas shown in table 5 are statistically the same, and since all three are larger than the corresponding value for the Bar males given in table 1, it seems probable that the true value for the disc area is closer to those of table 5 than that of table 1.

It will be noticed that the only age at which there is a significant statistical difference in disc size in both sexes between Bar and modified Bar is at 60 hours of larval life (table 1) and that contrary to expectation, Bar is larger than modified Bar. Table 3 shows that during the interval of 48 to 60 hours of larval life Bar has a faster growth rate than either wild type or modified Bar; while during the interval of 60 to 72 hours, Bar has a

slower growth rate than either wild type or modified Bar. Table 4 shows that the ratio of wild type to Bar at 60 hours is significantly smaller than that at 48 or 72 hours. Furthermore, only at 60 hours is there a significant difference between the ratios of wild type to Bar and wild type to modified

TABLE 4

Ratios of + eye discs to B and B;m(B) eye discs. (Calculated from the data of table 2.)

RATIO	AGE				
	36	48	60	72	84
$+/B$	1.15±0.05	1.57±0.05	1.24±0.01	1.31±0.03	1.42±0.04
$+/B;m(B)$	1.30±0.04	1.61±0.05	1.48±0.01	1.34±0.03	1.30±0.03

Bar. In addition, the growth curve of the Bar eye discs as plotted in figure 1 shows only one point very much out of line with that of the wild type—namely, that at 60 hours. All the other points fit fairly closely to a line drawn parallel to that of the wild type growth curve. For these several reasons it appears likely that the measurements of the Bar eye discs at 60 hours are too large—that is, that the larvae were actually physiologically older than 60 hours. This could easily be due to an undetected rise in temperature. Since there is no consistent size difference between the eye discs of Bar and modified Bar, the conclusion that they are identical in size and exhibit the same growth pattern seems warranted.

TABLE 5

Areas in square millimeters of the camera lucida drawings of Bar eye discs from mature larvae which had spent the first 24 hours of their development at 25°C and the remainder at the indicated temperatures. (Magnification=146×.)

TEMPERATURE	♀♀			♂♂		
	MEAN±σ_M	C_v	N	MEAN±σ_M	C_v	N
20°	1112.5±18.5	10.7	41	1057.5±28.3	14.2	28
25°	1104.0±19.8	11.0	37	1007.6±10.0	5.8	35
28°	1098.7±21.5	11.7	36	1021.8±17.7	9.6	30

The possibility remains, however, that the difference in facet number between Bar and modified Bar is not great enough to be detected as a size difference in the eye discs under the conditions of this experiment. The data of table 6 showing the measurements of eye discs taken from mature double Bar (BB) and infra Bar (B^i) larvae raised at 25°C clearly demonstrate that this is not so. These larvae were raised under exactly the same culture conditions as were the wild type, Bar and modified Bar larvae

TABLE 6

Areas of BB and B^i eye discs from mature larvae raised at 25° C. (Magnification = 146×.)

GENOTYPE	♀♀			♂♂		
	MEAN $\pm \sigma_M$	C_v	N	MEAN $\pm \sigma_M$	C_v	N
BB	931.5±18.5	12.9	42	948.4±18.7	12.5	43
B^i	1311.5±29.9	12.8	31	1168.9±21.5	9.8	28

except for temperature, which in this case was 25°C instead of 27°C. Measurements were made when approximately one-half of the larvae in the dish had pupated. When raised at 25°C, the *BB* stock used has 28 facets (STEINBERG in press). The *B* stock when raised at 27°C probably has between 50 and 60 facets as indicated by work on other *B* stocks with similar facet numbers to that of the stock used here (*cf.* Margolis 1935a). It is clear then that a decrease of approximately only 35 facets (or 50 percent) may result in a detectable decrease in disc size. The B^i eye discs are clearly larger than either the *B* or *B;m(B)* eye discs (table 1, 6). The B^i stock used here has 345 facets at 25°C (STEINBERG in press). Therefore, an increase of approximately 300 facets (400 percent) if Bar is taken as the base, or of approximately 125 facets (50 percent) if the modified form is taken as the base, may be easily detected as an increase in disc size. There remains, then, the fact that although Bar and its modified form differ by about 150 facets (200 percent), a number which we have seen may lead to a detectable difference in disc size, no difference was found.

Histological

ENZMANN and HASKINS (1938) studied the histological development of the eye discs of wild type larvae. They reported that at 26 hours (presumably from the time of egg laying, although this is not made clear in the paper; nor is the temperature stated) ". . . . the anlage appears to consist of rod-like elements each of which contains one to three nuclei." These elements increase in number, they report, as development proceeds until in older larvae ". . . . their number approaches that of the number of ommatidia in adult flies." It is perhaps worth noting that although the authors report the time of origin of these 'elements' to be 26 hours, in their table 2 they give the number of 'elements' present at 18 and 21 hours.

KRAFKA (1924) reported that histological examination of the eye discs of larvae 24 hours before they pupate showed that ". . . . the rudiments of the ommatidia are fixed. . . ." He found that ". . . . four terminal cells and six basal cells arranged around a deeply staining axis, form a cylindrical unit that is repeated over the entire inner surface of the imaginal disc." He concluded that it was this differentiation of the rudiments of the omma-

tidia which determined the end of the T.E.P. In view of the findings to be reported here, it is worth noting that although ENZMANN and HASKINS (1938) studied the same and later stages than KRAFKA, they did not report the finding of structures such as he reported.

Larvae were raised in Petri dishes at 25° and 27°C as recorded above. Groups of larvae were fixed at various ages after hatching from the egg. The fixatives used were Carnoy II (glacial acetic one part, chloroform three parts, and absolute alcohol six parts) at 50°C and hot aqueous Bouin (90° to 100°C). It was soon found that Carnoy and particularly a modification of Carnoy consisting of glacial acetic two parts, chloroform three parts, and absolute alcohol five parts gave much better fixation than did Bouin, and hence most of the work was done with Carnoy or the modification reported above. The larvae were sectioned at 5, 7, or 10 micra, the last being used most often. The slides were stained in either Gentian violet or Delafield's haemotoxylin with eosin as a counter stain.

Wild type eye discs

Figures 2 through 10 are photographs of representative sections from larvae of various ages. At 24 hours after hatching (27°C) the eye disc is leaf shaped (MEDVEDEV 1935, and fig. 2, 3, 4). Contrary to CHEN (1929), who reported that the optic stalk first begins to develop at 40 hours, the present study shows that at 24 hours a well developed optic stalk is present and that it is already attached to the brain (fig. 2, 3, 4; also *cf.* WEISMANN 1864, fig. 19b). KALISS (1939) has shown that the frontal sac is already present at 12 hours of embryonic life—that is, after one-half of the embryonic life is completed. This observation is supported by PATTERSON'S (1929) X-ray experiments in which mosaics of the entire eye or of one-half of the eye were obtained only when very early egg stages were treated. Consequently, it is not surprising that the optic stalk should be developed as early or earlier than 24 hours rather than as late as CHEN indicated.

The cephalic complex at this time (24 hours) is several cell layers thick. The cells are large, long, and fusiform. They overlay one another somewhat as do shingles on a roof. Beyond this there is no visible organization into a pattern. At 48 hours the antennal and optic discs are clearly formed as individual structures (fig. 6). They are connected by a thin layer of cells. There has been a tremendous increase in cell number, but no special organization of cells into larger units has occurred. In fact the cell arrangement in the eye and limb discs is remarkably similar (fig. 5, 6, 7).

By 72 hours at 27°C the discs have grown considerably. The antennal disc has undergone much folding as have also the wing and limb discs (see AUERBACH 1936 for a description of development of the wing and limb discs). The eye disc has increased greatly (table 1, 2). At this time

the organization of the cells into larger units may be seen for the first time. The units appear to consist of clusters of four cells. The clusters are regularly arranged in a pattern quite similar to that of the ommatidia. It is probably because of this that KRAFKA, who first saw these structures, believed them to be the forerunners of the ommatidia themselves. This may be true, but in the author's opinion, the question had best be left open until further study actually affords direct evidence in support of this assumption, particularly since BODENSTEIN (1938) claims that the ommatidia do not differentiate until after pupation. At 72 hours the clusters are relatively few in number, closely approximated to each other, and not very distinct. In 96 hour old larvae (temperature = 25°C; these larvae are physiologically the same age as 84 hour old larvae raised at 27°C) these units have greatly increased in number. They are now for the most part widely separated from each other although still arranged in very regular rows (fig. 8). That they are superficial structures with a thickness of about ten micra may be seen by a comparison of figures 8, 9, and 10 (these figures are photographs of three adjacent serial sections of the eye disc, figure 8 is the most superficial). That each unit is a cluster of four cells may be seen more readily in the older larvae (fig. 11). Thus far it has not been possible to confirm KRAFKA'S (1924) statement that 24 hours before pupation ". . . . four terminal cells and six basal cells arranged around a deeply staining axis, form a cylindrical unit that is repeated over the entire inner surface of the imaginal disc." The present study has not revealed any more than four cells in any cluster, nor has it shown a deeply staining axis. At no stage were any structures resembling those reported by ENZMANN and HASKINS observed.

Bar eye discs

Through the first 48 hours (temperature = 25°C) of larval life, the Bar eye disc is histologically the same as the wild type. The first difference was noted at 72 hours (temperature = 25°C) after hatching. Bar unlike the wild type showed no evidence whatsoever of the organization of the cells into clusters. Histologically the disc appears not to differ from one which is 48 hours old. What is even more startling is the fact that at 96 hours (temperature = 25°C) there is still no sign of any organization of the cells into the clusters described for the wild type (fig. 12, 13). Twenty-two larvae were examined at 72 hours and 23 at 96 hours. As yet no study of later stages (prepupal and pupal) has been made, consequently the possibility remains that the organization of the cells into clusters occurs later.

It was pointed out above that KRAFKA concluded that it was the formation of the clusters of cells ("rudiments of the ommatidia"—KRAFKA) that determined the end of the T.E.P. This appears entirely unlikely in view of the present observations. In Bar no differentiation was observed

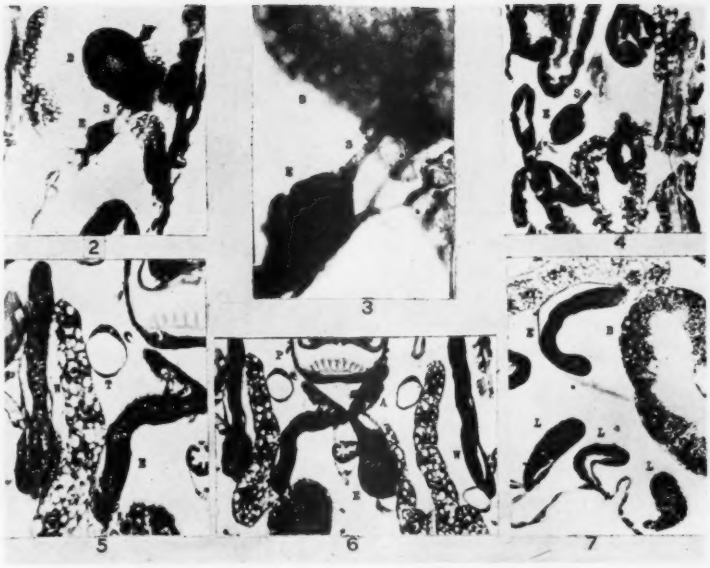


FIGURE 2.—Longitudinal section through brain and cephalic complex of a 24 hour old wild type larva showing the optic stalk extending to brain. Sectioned at 20μ. 300×.

FIGURE 3.—Same as figure 2. 1000×.

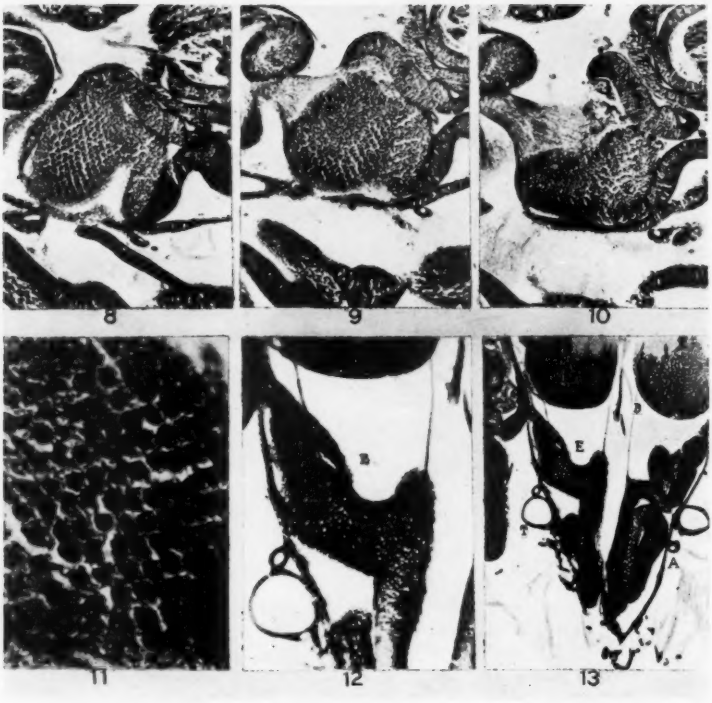
FIGURE 4.—Longitudinal section through another 24 hour old wild type larva showing cephalic complex and optic stalk. Sectioned at 10μ. 300×.

FIGURE 5.—Photograph of a portion of figure 6. 420×.

FIGURE 6.—Frontal section of a 48 hour old wild type larva. Compare eye and wing discs. 260×.

FIGURE 7.—Longitudinal section of a 48 hour old wild type larva. Compare eye and limb discs. 260×.

List of abbreviations used in figures 2 through 7: A antennal disc, B brain, E cephalic complex in 24 hour old larvae; eye disc in older larvae, L limb disc, P pharynx, S optic stalk, T trachea, W wing disc.



FIGURES 8, 9 and 10.—Successive sections through the eye disc of a 96 hour old wild type larva. 260×.

FIGURE 11.—Photograph of the central portion of the eye disc of figure 8 showing details of the structure of the cell clusters. 1000×.

FIGURE 12.—Photograph of a portion of figure 13. E eye disc. 420×.

FIGURE 13.—Longitudinal section of the brain and cephalic complex of a 96 hour old Bar larva. A antennal disc, B brain, T trachea. 200×.

in any larval stage, yet the T.E.P. ends about 24 hours before puparium formation (at 25°C). In wild type on the other hand differentiation into what KRAFKA has called "rudiments of the ommatidia" occurs at least as early as 72 hours after hatching, and probably earlier. MARGOLIS and ROBERTSON (1937) found that the T.E.P. in wild type extended over the entire larval period, up to prepupa formation. Regardless of what role these structures play in the development of the eye, it is certain that their formation does not determine the end of the T.E.P.

A more complete discussion of the role of these "cell clusters" in the differentiation of the eye especially with regard to the differences between Bar and wild type will have to be deferred until histological observations on prepupal and pupal stages have been made.

DISCUSSION

The study of the growth curves of Bar, modified Bar, and wild type eye discs showed that the discs of both of the former are already smaller than wild type at 36 hours after hatching, the earliest stage examined, and that from this point on they grew at the same rate as wild type. Since this age is prior to the T.E.P. at 27°C, it is obvious that Bar changes the size of the eye discs before the T.E.P. Furthermore, since the growth rate of the Bar eye discs from 36 hours until puparium formation is the same as that of the wild type, it must be concluded that Bar does not affect growth during this period. The data, however, do not offer any direct evidence on whether or not Bar affects the growth rate prior to this period.

KALISS (1939) has shown that the frontal sac is present when only approximately one-half of the embryonic period is completed. Therefore, at 27°C the frontal sac—cephalic complex—has been present and growing for about 42 hours by the time 36 hours of larval life are completed. Consequently, it is possible that the growth rate of Bar is lower than that of wild type prior to 36 hours of larval life and the same as that of wild type after this point. If this is true for Bar, it would also be the likely explanation for the mutants studied by MEDVEDEV (1935)—that is, eyeless^2, glass^2, and Lobe^e. But at 36 hours of larval life the eye discs of these mutants are all different in size from each other. There are several possible explanations to account for the origin of these differences in disc size. It may be assumed that each mutant exhibits a different growth rate from all the others during this period; or that they all have the same growth rate during this period, slower than that of the wild type, and that each maintains this reduced rate for varying periods of time, and henceforth grows at the same rate as the wild type. Such shifts in growth rates as are assumed in these two alternatives are, to the author's knowledge, unprecedented and seem to be quite unlikely.

The difference in disc size might have arisen through the later origin of the cephalic complex of the mutants as compared to wild type. No histological study of the early embryology of the mutant forms has as yet been made, consequently no direct evidence can be brought to bear on this question. The observation that although differentiation has occurred in the eye discs of wild type by 72 hours and is extensive at 96 hours (fig. 8, 9, 10) none has occurred in Bar (fig. 12, 13) would seem to argue in favor of this assumption. However, if the cell clusters are really the precursors of the ommatidia as KRAFKA assumed, one would expect many fewer in Bar than in wild type; hence it might be possible that, although present, they were missed. Only further study can answer this question. But if this explanation is invoked to explain the size difference of the four mutants (*B*, ey^2, gl^2, and L^c), we are faced with the necessity of assuming that the developmental pattern of *Drosophila melanogaster* is an extremely labile one so that the relative time of origin of a structure may be shifted very easily. In view of the known mosaic nature of the Dipteran egg (see RICHARDS and MILLER 1937 for review) and in view of the many experiments on amphibia showing the non-labile nature of the time relationships in the developing organism, this does not seem likely.

A third possible explanation is that the capital of cells involved in the initial formation of the cephalic complex is different in the various mutants and that the growth rate is the same in all the mutants throughout development. This explanation does not involve any of the difficulties inherent in the first two. The necessity for an extremely large number of different growth rates (proportional to the number of mutants affecting eye size) maintained for varying lengths of time is obviated, as is the necessity for assuming that the time of origin of the cephalic complex relative to the general growth pattern is so labile as to be almost haphazard. In addition, there is abundant evidence to show that the initial cell number of an organ does vary. Finally, the growth curve data on Bar, modified Bar, $eyeless^2$, $glass^2$, and $Lobe^c$ indicate that the growth rates of the eye discs of all these mutants, at least during that part of the egg-larval period when they can be measured, are identical with each other and with wild type. For these reasons, the assumption that Bar reduces the size of the eye discs and ultimately the facet number by reducing the initial capital of cells forming the cephalic complex seems the most likely.

The work on the T.E.P. shows that the final facet number is not completely determined at the time of origin of the eye anlage but that it may be varied considerably by changes in temperature during a specific period of larval development. The above data and discussion indicate that the T.E.P. is not the period during which Bar acts to reduce facet number (KRAFKA, and others). It is probable that the T.E.P. is simply the period during which the facet number may be influenced by temperature.

Before discussing the data further, it will be necessary to review some facts of the development of the imago.

WEISMANN (1864), in his remarkable study of the imaginal discs of *Musca vomitoria*, showed that the eye disc gives rise to a good deal of the head in addition to the eye. BEADLE and EPHRUSSI (1936) and CHEVAIS (1937) have shown this to be true for *Drosophila melanogaster* as well. Experiments of the author in which the antennal and optic discs of mature larvae were separated before transplantation into larvae of the same age show that in addition to the eye, the eye disc forms all of the head with the exception of the antennae and the mouth parts. STURTEVANT (1927) has pointed out that the region which is not faceted in the Bar eye but is so in the wild type is chitinous and appears exactly like the rest of the chitinous head.

The experiments of HOWLAND and CHILD (1935) in which eye and head defects in Drosophila imagos were obtained as a result of injuries to a localized region in four hour old eggs, and those of PATTERSON cited above, support the assumption that the cephalic complex is determined very early in embryonic development. KALISS's (1939) observation that the frontal sac is present when only one-half of the embryonic development is completed lends further support to this assumption.

Direct evidence that the cephalic complex is determined after 24 hours of larval life was afforded by experiments in which the cephalic complex was transplanted from larvae of this age to 48 hour old hosts. Four sets of transplants were made as follows: wild type into wild type, wild type into Bar, Bar into wild type, and Bar into Bar. In every case the implant was autonomous (table 7). The wild type cephalic complex gave rise to a wild

TABLE 7

Transplants of 24 hour old eye discs into 48 hour old hosts. Temperature=27±1° C.

GENOTYPE		PHENOTYPE OF	
DONOR	HOST	IMPLANT	N
+	+	+	2
B	*B*	*B*	6
+	*B*	+	2
B	+	*B*	5

type eye regardless of the host and likewise the Bar cephalic complex gave rise to a Bar eye regardless of the host. The other structures formed by the cephalic complex (see above) were also shown by these experiments to have been determined at the time of transplantation. These experiments indicate not only that the eye is determined by 24 hours of larval development, but also that the Bar eye disc is already determined to form eye, but more specifically Bar eye. This conclusion is supported by the data

on the growth curve which indicate an early effect of Bar on the eye disc. What remains undetermined is just what size of Bar eye will be formed.

As pointed out in the introduction to this paper, it has been shown that an increase in temperature leads to a decrease in facet number in Bar flies and that temperature is effective during only a limited period of the larval stage. It was also pointed out that these facts lead to a hypothesis which assumed the existence of some mechanism inhibiting facet formation and which is effective during the T.E.P. This mechanism, it was supposed, had a higher Q_{10} than that of the general growth processes of the organism, and hence at higher temperatures fewer facets were formed.

STURTEVANT (1925) reported the occurrence of an allele of Bar, infra-Bar (B^i) which had a facet number intermediate to that of Bar and wild type. LUCE (1926, 1931, 1935) studied the effects of temperature on this mutant. He found that contrary to what was found for Bar, an increase in temperature yielded an increase in facet number. Here again as in Bar, temperature was effective during only a limited period of the larval stage. To explain these facts, Luce assumed that in B^i as in B there is a process inhibiting facet formation, but that unlike the process in Bar, this process has a lower Q_{10} than that of the general growth processes of the larva.

For Bar, at any rate, it seems highly unlikely that the postulated "facet inhibiting reaction," if it exists at all, is responsible for the major reduction in facet number, unless its time of action be assumed to be at a stage much earlier than that of the T.E.P. But this is unjustified, since the "facet inhibiting reaction" was invoked to explain the T.E.P. It is possible of course to retain the "facet inhibiting reaction" as an explanation of the T.E.P., but to assign a secondary role to it—that is, to have it function in the determination of the final size of the Bar eye, but not in deciding whether the eye will be the wild type or Bar.

The development of the Bar eye may be pictured as follows: by 24 hours of larval development, and in view of the mosaic pattern of the Dipteran egg and of the early origin of the frontal sac, very likely much earlier than this, the phenotype of the Bar eye is already determined; this determination, however, is not complete but labile so that the facet number may be shifted, within limits, to either a greater or smaller number. Several factors are already known which do this. Temperature is of course the most commonly known and used; others are pure oxygen atmosphere (MARGOLIS 1939) and a nitrogenous extract of Calliphora larvae when fed to Bar larvae (EPHRUSSI, KHOUVINE, and CHEVAIS 1938; CHEVAIS and STEINBERG 1938). It is noteworthy that both these agents are effective at times other than that at which temperature is effective. Facet number may be affected by increasing or decreasing the length of the growth period of the eye disc relative to that of the larva in which it arose by transplanting the

disc into a younger or older host (BEADLE and EPHRUSSI 1936; BODENSTEIN 1939). The present discussion, however, is concerned only with those cases in which the disc remains in its normal position.

As pointed out above, this shifting of the facet number is possible because determination is not complete until late in development. The labilely determined tissue may form either head chitin or eye (facets, ommatidia). If this be correct, an increase or decrease in facet number in the Bar eye caused by a change in temperature should not involve a change in the size of the eye disc. The following experiment was performed to test this assumption. Eggs from genotypically Bar females were collected over a 24 hour period at 25 ± 0.25°C. Newly hatched larvae were collected over two hour intervals. They were permitted to continue the first 24 hours of their development at 25°C. At the end of this time the larvae were divided into three groups: one group was transferred to 28 ± 1°C, a second to 20 ± 1°C, the third was left in the 25°C incubator. The larvae were permitted to continue their development at these temperatures until approximately one-half the larvae in a particular Petri dish had pupated. In this way a uniform physiological age was obtained. The eye discs of the remaining larvae were then measured as described above. Because the entire T.E.P. was spent at the temperature at which the larvae had completed their development, the facet numbers of each of the groups should correspond to that characteristic for the temperature at which development was completed. Facet number should vary, therefore, from approximately 50 (corresponding to 28°C) to approximately 125 (corresponding to 20°C), a range sufficiently large to yield disc size differences as was shown above. Table 5 shows the data derived from the measurements of the areas of these eye discs. There can be no doubt that the data show no significant difference between the areas of the eye discs of either sex. The eye discs of the females vary in size from 1098.7 ± 21.5 to 1112.5 ± 18.5; those of the males vary from 1007.5 ± 10.0 to 1057.5 ± 28.3. The difference between these two latter values is 49.9 ± 30.0 and is not significant. Furthermore, it should be noted that in the males the area at 25°C is smaller than that at 28°C, although not statistically so (table 5). The data, therefore, are in agreement with what was predicted by the hypothesis and to that extent confirm the hypothesis.

The modified Bar eye discs were shown to be identical in size with the Bar eye discs throughout the period of observation despite the fact that the facet number of the former is considerably higher than that of the latter. It will be recalled that $m(B)$ is a modifier of Bar which has no phenotypic expression in the absence of Bar or one of its alleles. It has been shown for at least one extrinsic modifier of Bar—namely, temperature—that a change of facet number brought about through its action

does not affect the disc size. There is no *a priori* reason for assuming that intrinsic modifiers of Bar may not act in the same way—that is, shift labilely determined tissue in one direction or another so as to increase or decrease the facet number. It is proposed that $m(B)$ is an intrinsic modifier of facet number in Bar which acts by causing more of the labilely determined tissue to form facets than would have done so in the absence of $m(B)$. The explanation of how the modifier causes this result must await further experimentation.

It is indeed a pleasure to acknowledge my indebtedness to DR. L. C. DUNN for his many helpful suggestions and for his sustained interest during the progress of these experiments.

SUMMARY

The growth curves of Bar, of Bar in association with a second chromosome modifier of Bar ($m(B)$), and of wild type eye discs were determined from 36 hours after hatching until, but not including, puparium formation.

All three eye discs showed the same growth rate throughout the period of observation.

Bar and modified Bar were the same size throughout the experiments. Both were smaller than wild type at 36 hours, the earliest stage measured. This is before the onset of the T.E.P.

Histological examination of the eye discs showed the presence in the wild type eye discs of 'cell clusters' consisting of four cells each at 72 hours of larval life (temperature = 25°C) and indicated that these increased in frequency between 72 and 96 hours. No such 'cell clusters' were found in Bar even at 96 hours.

The time of origin of the optic stalk was placed at no later than 24 hours after hatching (temperature = 27 ± 1°C).

Some possible explanations of the mode of development of the Bar eye were discussed. The following seemed the most likely: the Bar eye was determined very early in development. This determination was labile so that facet number could be changed, within limits, by extrinsic and intrinsic modifiers. Changes in facet number brought about in this way would not affect disc size. This was tested for temperature and shown to be true.

It was assumed that the $m(B)$ is an intrinsic modifier of Bar which affects the distribution of the labilely determined tissue in such a way that more of it goes to form facets than in the absence of $m(B)$.

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AN ANALYSIS OF THE EXPRESSION OF THE MUTANT "ENGRAILED" IN DROSOPHILA MELANOGASTER[1]

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INTRODUCTION

THE fields of embryology and genetics find a common meeting ground in the study of the manner in which the genes control the development of an organism. Through analyses of the deviations from normality brought about by any specific mutant gene it is possible to obtain some idea of the processes of development in which it is involved and, therefore, probably also of those in which its wild type allele has had a part.

The mutant "engrailed" in *Drosophila melanogaster* seemed well suited for such an analysis for the following reasons. (1) The variability associated with each of the principal phenotypic effects of engrailed could be investigated experimentally since each is sensitive to environmental changes. (2) One of the effects of engrailed is rather unusual in that it produces a specific structure, a sex-comb, in a region of the tarsus where normally no such organ exists. Accordingly two sex-combs are found on one leg, the normal one and the mutant supernumerary. Thus the behavior of the two under various environmental conditions could be compared. (3) Variations in the sex-limited nature of the extra sex-comb could be observed under conditions which affect the normal sex-comb or sex itself.

There are several striking effects of engrailed, all of which are variable in their expression. (1) As just mentioned, there is an extra sex-comb in the male which is smaller than the wild type sex-comb and which is placed in a mirror image position to it on the outside of the first tarsal joint of the foreleg. Its size, as measured by the number of teeth, has been observed to vary from 0 to 11 teeth. (2) There is a median cleft in the scutellum which shifts from a flattening of the normally pointed posterior border to a complete separation of the two halves. (3) The wings are shorter, broader, and thinner than in the wild type. The venation is disturbed, with large or small gaps in the fourth and fifth longitudinal veins and an irregular branching plexus of extra veins. (4) Since this work was completed, a further effect of engrailed has been demonstrated by MRS. V. S. CURRY at the CALIFORNIA INSTITUTE of TECHNOLOGY (unpublished). This is a rotation of the genitalia in males. Males with extremely rotated gen-

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italia are sterile, and hence in homozygous engrailed stocks there is selection for modifiers suppressing this effect. By making a balanced, that is heterozygous, stock MRS. CURRY is able to obtain homozygous engrailed males all of which show rotation.

Engrailed was first described by EKER (1929). It is an autosomal recessive gene, located in the second chromosome at locus 62.0. EKER describes the scutellar and wing modifications but makes no mention of the extra sex-comb. In his text figure of an engrailed male there seems to be such a sex-comb depicted on the left foreleg but not on the right. Its presence was discovered during a routine transfer of stocks (C. STERN, September 1937). This finding was confirmed by examination of the engrailed stock at the CALIFORNIA INSTITUTE OF TECHNOLOGY by the late DR. C. B. BRIDGES, and also by examination of a similar stock from the Department of Genetics of the CARNEGIE INSTITUTION OF WASHINGTON at Cold Spring Harbor, N. Y. In the experimental work reported in this paper the latter stock was used. It was homozygous for the second chromosome recessive "purple" (*pr*, 2–54.5) as well as engrailed (*en*).

FIGURE 1.—First tarsal joint of engrailed male showing primary (above) and secondary (below) sex-combs. Camera lucida drawing, 223×.

EXPERIMENTAL WORK

Sex-Combs

In Drosophila the sex-comb is a secondary sex character limited to the male. It consists of a row of modified bristles in the shape of a comb with heavy black teeth and is located on the inside (mesial side) of the first tarsal joint of the foreleg at its distal end.

The sex-combs of engrailed will henceforth be designated as primary and secondary. Primary refers to the sex-comb normally present on the mesial side of the foreleg; secondary to the extra one on the lateral side. They are shown in figure 1. The arrangement of the teeth in the primary sex-combs is almost invariably a straight row, but is much less regular in the secondary. If the number of teeth in the latter is small—from 1 to 5 or 6,—they are usually regularly placed, but as the number increases the arrangement becomes less uniform. Some representative types of sex-combs are shown in figure 2.

STURTEVANT (1929) has described a similar mutant in *D. simulans*, called "bipectinate." It is characterized by an extra sex-comb on the second tarsal segment, resembling the normal condition in *D. pseudoobscura* (see below). This mutant, like engrailed, is sex-limited in the expression of the extra sex-comb. Also like engrailed, it has other phenotypic effects which are not sex-limited—absence of one tarsal segment on every leg and changes in the shape of the wing.

It is interesting to note that features pertaining to sex-combs are of taxonomic importance. They are found in many, but not all, species of Dro-

FIGURE 2.—Various types of sex-combs. A, right primary, B, left secondary; C and D, left and right secondaries, respectively (19°), showing irregular arrangement of teeth. Camera lucida drawing, 223×.

sophila, and if present, they are characteristic in location, size, and form for the particular species. In species which possess them there is always a sex-comb on the first tarsal segment. A number of species normally have a second sex-comb on the inside of the second tarsal joint. The occurrence of this distal sex-comb is recorded below (STURTEVANT 1921, STURTEVANT and DOBZHANSKY 1936, KIKKAWA and PENG 1938).

SPECIES GROUP	DISTAL SEX-COMB
melanogaster	Present, except in *melanogaster*, *simulans*, and *bipectinata**
affinis	Only 1 tooth
obscura	Present

* *D. bipectinata* has two sex-combs, one immediately behind the other, on the inside of the first tarsal joint.

MATHER and DOBZHANSKY (1939) made a statistical analysis of the number of teeth in the sex-combs in the two races of *D. pseudoobscura*. They found that the distal sex-comb had fewer teeth than the proximal and demonstrated a significant racial difference in the mean number of teeth in corresponding sex-combs, as well as a significant difference between some strains of the same race. They showed that if suitable statistical techniques are used, the number of teeth in the sex-combs may be employed as a taxonomic character to differentiate the two races with 90 percent of accuracy.

Effect of temperature upon the sex-combs

Methods.—In order to provide optimal developmental conditions, the flies used in the temperature experiments were bred in the following manner. Parent flies were placed, without etherization, in a fresh bottle of standard cornmeal-molasses-agar culture medium which had been well seeded with yeast. After 24–48 hours they were transferred to bottles containing small dishes of yeasted cornmeal-agar and allowed to oviposit until about 125 eggs were present. Then the dishes were removed and new ones put in their place. The number of eggs on each dish was counted and the entire contents of the dish transferred to a 10.5 cm fingerbowl containing a layer 1–1.5 cm thick of freshly yeasted cornmeal-agar. The number of eggs in each fingerbowl ranged from 110 to 135, averaging 125. A larger number was not used because of the possibility of overcrowding the developing larvae.

The fingerbowls containing eggs were placed in incubators at temperatures of 14°, 19°, 25°, and 29.5°C. The incubators were of the general type

TABLE 1

*Mean number of teeth in sex-combs of engrailed.**

TEMP.	N	PRIMARY		SECONDARY	
		MEAN	σ	MEAN	σ
14°	180	10.42±0.10	0.96±0.07	6.26±0.16	1.50±0.11
19°	648	10.51±0.05	0.92±0.04	5.71±0.11	1.88±0.08
25°	622	11.04±0.06	1.01±0.04	3.57±0.11	1.86±0.08
29.5°	566	10.92±0.06	1.01±0.04	0.13±0.02	0.34±0.02

* These data approximate a normal distribution curve; therefore the mean and standard deviation are a legitimate form of analysis of them.

described by BRIDGES (1932) and maintained a constant temperature with no greater variation than ±0.2°. When the larvae pupated, they were placed in half-pint culture bottles and allowed to hatch at the same temperature at which they developed.

As a quantitative evaluation of the size of the sex-combs the number of teeth present was determined in the following way. The forelegs were removed under a binocular dissecting microscope and placed on a dry slide. The teeth were counted under the high power of a compound microscope. Right and left sex-combs were recorded separately, but since analysis showed no significant difference in their mean sizes, the data were combined in computing the average number of teeth at each temperature. The mean and standard deviation together with their standard errors were calculated for both primary and secondary sex-combs. Differences between means were tested by the formula

$$\epsilon_d = \sqrt{\frac{\sigma_1^2}{N_2} + \frac{\sigma_2^2}{N_1}}$$

in which ϵ_d is the standard error of the difference, σ_1 and σ_2 are the standard deviations of the two means, and N_1 and N_2 are the two populations. If the observed difference was more than three times this value, it was considered significant.

Results.—The experimental results obtained by this procedure are presented in table 1. The primary sex-combs do not show a great deal of variation. Below are given the differences between their means and the standard errors of these differences. It may be seen that there is a significant difference between the high and low temperature groups but no such difference between the two members of each group.

TEMPERATURES	d	ϵ_d
14°–19°	0.09	0.11
*14°–25°	0.62	0.11
*14°–29.5°	0.50	0.12
*19°–25°	0.53	0.08
*19°–29.5°	0.41	0.08
25°–29.5°	0.12	0.08

* Significant.

These results fall in line with those of several investigators who have worked with both wild and mutant stocks in which only the normal, or primary, sex-comb was present. They found that the number of teeth is rather constant. There is a small amount of variability, but its range seems to be about the same under different environmental conditions. HOGE (1915) was able after seven generations of selection to produce a race which had a higher average number of teeth than the unselected controls. EIGENBRODT (1930) observed the effects of five different temperatures upon the number of teeth in the sex-combs of a homozygous forked Bar stock. He found no consistent or significant alteration in the number of teeth. COMBS (1937) made a further study of the effect of temperature on the sex-combs, using several mutant stocks and two temperatures. For all the stocks he found a slightly but significantly higher mean number of teeth at 27° than at 17°. The stability of the primary sex-comb is thus maintained rather well in a variety of conditions. Neither temperature nor selection can very effectively alter its size. However, the fact that there are significant differences between the number of teeth in the sex-combs of different homozygous stocks shows that genetic factors whose more obvious effect is not on the sex-comb nevertheless do influence the size of this structure.

In contrast to the results obtained for the primary sex-combs is the decided response to temperature exhibited by the secondary sex-combs. A decrease in tooth number is associated with an increase in temperature. At the three lower temperatures a large amount of variability is found—from 2 to 9 teeth at 14°, from 0 to 11 at 19°, and from 0 to 10 at 25°. At 29.5° there is a striking loss of secondary sex-combs—in 81.3 percent of the individuals studied they are completely absent. Only one fly was found with as many as three teeth in one of its secondary sex-combs. Below are given the differences, with their standard errors, between the mean tooth number at the various temperatures. Obviously all are significant.

TEMPERATURES	d	ϵ_d
14°–19°	0.55	0.15
14°–25°	2.69	0.15
14°–29.5°	6.13	0.07
19°–25°	2.14	0.11
19°–29.5°	5.58	0.08
25°–29.5°	3.44	0.08

In order to determine whether or not the variability in the number of teeth in the sex-comb on one foreleg was in any way connected with the variability on the other foreleg, correlation coefficients (r) were calculated separately for primary and secondary sex-combs on the right and left sides (table 2). None of the coefficients is high, and some are not significant, being less than three times the standard error of r. Even the highest of

TABLE 2

Coefficients of right-left correlation between the number of teeth in the primary and secondary sex-combs.

SEX-COMB	14°	19°	25°	29.5°
Primary	0.01±0.11	0.38±0.05	0.04±0.01	0.15±0.06
Secondary	0.23±0.10	0.41±0.03	0.24±0.05	0.19±0.06

these—namely, 0.41±0.03 for the secondary sex-comb at 19°—indicates that no more than 16 percent of the variance between the two sides is due to factors common to both sides. The remaining 84 percent is random. Thus on the whole the two sides of the fly seem to vary independently with regard to the number of teeth in the sex-comb. Similar low positive correlations between the right and left sex-combs in races of wild type *D. melanogaster* were found by CARPENTER and BARROWS (CASTLE et al. 1906).

In his study of the bristle pattern of the mutant Dichaete in *D. melanogaster*, PLUNKETT (1926) points out that a low value of r is obtained when

there are uniform environmental conditions and genetic homogeneity. High correlations, on the other hand, will be given by stocks with numerous genetic differences or by homogeneous stocks when environmental influences are diverse. The low correlation coefficients in table 2 show that in those cases there must have been genetic and environmental uniformity; where the coefficients are higher it may be assumed that the external environment has played some role in effecting that correlation, although some genetic variability cannot be entirely excluded.

Of particular interest from a developmental point of view is the question of the correlation between primary and secondary sex-combs on the same foreleg (table 3). Again the values are all low or insignificant. They indicate that there is no associaton between the sizes of the two sex-combs on same leg, except possibly at 19°. PLUNKETT (1926) found a clear degree of association in Dichaete between the bristles on one side of the fly. However, in other mutants the situation is similar to engrailed where the association

TABLE 3

Coefficients of correlation between the number of teeth in the primary and secondary sex-combs on the same side.

SIDE	14°	19°	25°	29.5°
Right	0.15±0.10	0.14±0.05	−0.06±0.06	−0.06±0.06
Left	0.07±0.10	0.17±0.05	−0.06±0.06	−0.02±0.06

is lacking or doubtful. This has been demonstrated for scute-1 by CHILD (1935) and IVES (1939) and for polychaetoid by NEEL (1940b). These investigators concluded that each bristle locus behaves independently of of the others.

The most important point brought out by these studies of the correlations between sex-combs is the high degree of independence of each of the four sex-combs on one engrailed fly. This also agrees with the different effects which changes in temperature had upon them—the primary varying little and increasing in size with increase in temperature, the secondary varying a great deal and decreasing in size with increase in temperature. This point will be considered in more detail later when the manner of action of the engrailed gene is discussed.

Relation of body weight to sex-combs

There is one general effect of temperature which is not often taken into account in investigations of temperature effects on various phenotypic characters—its effect on the body size of the fly. It is a common observation that flies reared at high temperatures are in general smaller than those reared in a cooler environment. NEEL (1940a) attacked this problem

of the relation of body size to character expression in the mutants Dichaete and polychaetoid of *D. melanogaster*. To determine whether the expression of engrailed was bound up with body size as it was with temperature, an analysis of these relations was undertaken. CASTLE et al. (1906) had already reported an increase in the number of teeth in the wildtype sex-comb with increase in size, using tibia length as an index of the latter.

Methods.—Flies of various sizes reared at the same temperature were obtained, using the eggdish-fingerbowl technique with different population densities (125, 300, 600, or 900 eggs in a fingerbowl). Another method employed was the rearing of 100 eggs on the amount of food contained in an eggdish (about 15 cc versus 125 cc in a finger bowl). Flies reared under crowded conditions on a limited amount of food were at a lower nutritional level than those less crowded and hence were smaller. The temperatures used were again 14°, 19°, 25°, and 29.5°.

When the larvae had pupated, they were placed in vials which contained a few cc of food. The adults usually hatched over a period of several days, especially from the more crowded cultures. Each day those which had emerged were collected and put into freshly yeasted culture bottles. There they were allowed to feed freely for 36–48 hours. The males were then etherized and weighed in groups of 50–200 on an analytical balance and the average weight for each group computed. Sex-comb counts were made for each size group.

Since body weight is closely and positively correlated with tibia length (NEEL 1940a), it may be used as an index of fly size, even though its use may be attended by a fairly large error. The chief cause of this error is the amount of food present in the gut, which in turn is determined by the inclination of the fly to feed, the availability of food, etc. Every effort was made to obtain as uniform and optimum conditions as possible.

Linear plots were made of the average weight and mean sex-comb tooth number of each group of flies, and a straight line was fitted to the points for each temperature by a modification of the least squares method which permitted a minimizing of the squared perpendicular distances from the points to the line and thus afforded the most correct fit. Each point represents flies grown under the same conditions. If successive hatches from the same population unit differed in average weight, the mean for the whole group was computed. Each point (with two exceptions) was calculated from more than 100 flies, hence 200 sex-combs, and many groups were still larger.

Results.—Primary sex-combs.—The data for the primary sex-combs are plotted in figure 3. A considerable range of body weights was obtained and, as might be expected, the smaller flies were found at the higher temperatures. Actually the group with the highest body weight was obtained at

19° instead of 14°, and the smallest at 25°, not 29.5°. In the latter case the technical difficulties in rearing flies at 29.5° reduced the population to less than half the size of that at 25°. Consequently the sampling error of mean weights may be large. At any rate the difference in weight between the smallest flies at 25° and the smallest at 29.5° is only 6 percent. In the former case it has been pointed out (EIGENBRODT 1930) that as incubation temperature decreases weight increases down to about 17°. Below this

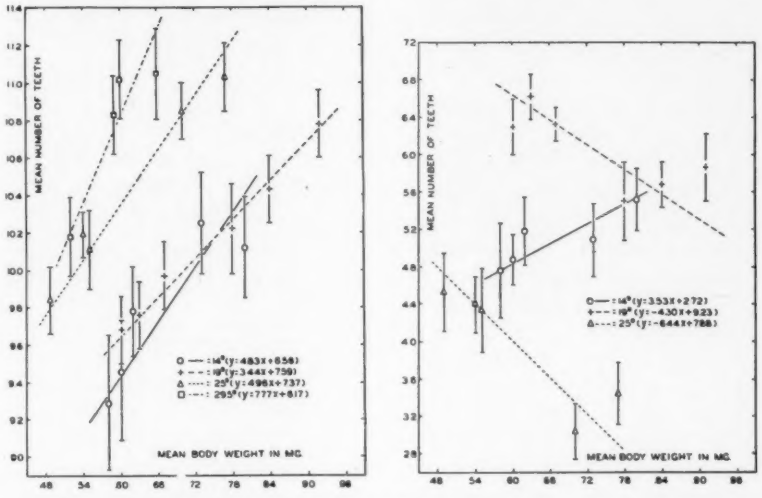

FIGURE 3.—(left) Relation of mean body weight to mean number of teeth in primary sex-combs at four temperatures. The bars extending from each point show the extent of three times the standard error of mean tooth number. Equations are for the regression of mean tooth number (y) on mean body weight (x).

FIGURE 4.—(right) Relation of mean body weight to mean number of teeth in secondary sex-combs at three temperatures.

there is very little increase in weight. Similarly RIEDEL (1934) has shown that the tibia length of wild type flies increases with a temperature decrease until about 15–16°. Further reduction in temperature causes a decrease in tibia length. NEEL (1940a) obtained larger flies of the Dichaete mutant at 19° than at 14°.

At each temperature there is a range of sex-comb sizes corresponding to the body weights. The fit of the straight lines which have been plotted on the graph is an indication that the relationship of the size of the primary sex-combs to that of the whole organism approximates a rectilinear one. The vertical bars extending from each point show the extent of three times the standard error of the mean sex-comb tooth number. In all cases these

bars touch the fitted line. The equations on the graph are of the general form $y = ax + b$ and are specifically for the regression of the mean number of teeth in the sex-comb (y) on average body weight (x) at each temperature. It may be seen that the slope (a) of the lines is steepest at the higher temperatures, while the intercepts (b) are all rather near together.

An evaluation of the relationship of body size to the temperature effect must now be made. It is obvious from figure 3 that while there is a definite correlation between body weight and sex-combs within each temperature, this relationship does not hold for the same size of fly at different temperatures. For example, let us examine flies which weighed on the average 0.60 mg each. At 14° they have an average of 9.46 teeth in their primary sex-combs, at 19°, 9.68 teeth, and at 29.5°, 11.02. Between 14° and 19° there is not a great deal of evidence for a temperature effect distinct from size, but the difference between these two temperature groups and that at 29.5° is considerable and significant ($\epsilon_d = 0.08$ for 19–29.5°). Other similar average weights at different temperatures give the same picture. A comparison may also be made by calculating from the regression equations for each temperature what the tooth number would be at a given size—namely, 0.60 mg. The values are as follows:

TEMPERATURE	TOOTH NUMBER
14°	9.44
19°	9.65
29.5°	10.83

There is clearly a difference in tooth number as the temperature increases. If size were playing any large role in the observable temperature effect on the primary sex-combs, we should expect all four lines in figure 3 to lie as near or nearer together as those for 14° and 19°. Their lying apart from each other indicates that sex-comb differences at various temperatures are largely independent of body size, most strikingly so in the higher part of the temperature range. If size were constant at all temperatures, the apparent effect of temperature would be considerably greater.

NEEL (1940a) has shown in his study of the correlation of body size with the expression of the mutants Dichaete and polychaetoid that in both cases there is a direct relationship between body weight and the number of dorsocentral bristles per side per fly. In his evaluation of the separate roles of size and temperature in the determination of bristle number he found for Dichaete that when size is held constant over a 15° temperature range, there is no consistent change in bristle number. Hence body size is an important factor in the temperature effect on the expression of this mutant. For polychaetoid, however, he found that the role of the size factor

is not the same for all temperature intervals but is greatest at high temperatures and least at low. It has now been demonstrated for engrailed that the size factor in the determination of the number of teeth in the primary sex-comb is important only at the lower temperatures.

Secondary sex-combs.—The secondary sex-combs present a strikingly different picture from the primaries. Instead of the uniformly positive relations for weight-tooth number there is a positive slope of the line at one temperature and negative slopes for the others. This is shown in figure 4. Data for 29.5° are not given, since secondary sex-combs are rarely present at this temperature. At 14° the data show as good an approximation to linearity as at any of the temperatures in figure 3. The slope is not so great and the intercept is smaller. At 19° and 25° there is obviously another sort of process involved as judged from the negative slope of the curves. The straight lines are clearly not a correct expression of the relationship of size to secondary sex-combs at these temperatures. There is a decided drop after an initial high tooth number for the smaller size groups, followed by a slight rise—whether significant or not is difficult to say, but the data at 19° strongly suggest that it is.

A comparison between the sizes of the secondary sex-combs in flies of the same body weight at different temperatures may be made as for the primary sex-combs. Flies which weighed 0.77 mg at 25° had an average of 3.45 teeth in their secondary sex-combs; flies weighing 0.78 mg at 19° had a mean of 5.50 teeth ($\epsilon_d = 0.13$). Between some other points in figure 4 there are not such great differences, but in these cases—namely, small flies at 14° and 25° and large individuals at 14° and 19°—the two temperatures involved behave differently in their weight-tooth number relationships. Comparisons have not been made between values calculated from the regression equations for 19° and 25°, since the lines are only very poor approximations to the data.

The correlation of increased size of an organ with decreased size of the whole organism is not a common one. Two other such cases, however, have recently been described for *D. melanogaster*. BODENSTEIN (1939) starved larvae of a stock carrying the mutant Bar in order to ascertain the correlation of increased larval life with the number of facets in the eye. The starved flies had a smaller tibia length and hence a smaller body size than their normal controls but the males had a much higher facet number. Body size is probably not the decisive factor here, since when larvae were completely starved they pupated earlier than the controls and had a lower facet number. CHILD (1939) working with the vestigial mutant also used starvation to increase the time of development at a constant temperature and obtained flies which were smaller but which had larger wings than the unstarved controls. However, it should be pointed out that the

production of vestigial wings is accomplished by a degeneration of the wing tissue rather than by a failure of it to develop. Thus this case is probably not comparable to the former ones.

Effect of intersexuality on the sex-combs

In attacking the problem of the sex-limited expression of engrailed it was sought to bring this gene into a genetic environment which would modify the sex of the individuals concerned. Such an environment is found in intersexes—individuals which have both male and female characteristics. It is known that sex-combs are present in most wild type triploid intersexes (2X+3A), but no data were available as to their size in intersexes compared to wild type diploid males. It is of interest to discover whether the secondary sex-comb occurs in intersexes homozygous for engrailed or whether the departure from absolute maleness suppresses or modifies the appearance of this sex-limited structure, and also whether the degree of intersexuality influences the expression of either or both primary and secondary sex-combs.

As a group intersexes are not uniform in appearance, especially in respect to the genitalia. Some show predominantly male structures, others female, and still others have neither typical male nor female parts. DOBZHANSKY (1930) has grouped intersexes into five classes, as follows: class 1, extreme male type; external male genitalia present; penis and genital arch symmetrical; class 2, external male genitalia or rudiments present; penis and genital arch asymmetrical, reduced in size or rudimentary; class 3, neither male nor female external genitalia present; class 4, external female genitalia present at least in rudimentary form; vaginal plates asymmetrical; class 5, extreme female type; external female genitalia present; vaginal plates symmetrical. He has shown that the type of intersex is influenced by genetic modifiers, since by selection for male or female type he could establish lines in which the percentage of either type was high with a corresponding decrease in percentage of individuals of the opposite type.

Intersexes homozygous for purple engrailed were obtained by repeated backcrossing of wild type triploid females to engrailed males. A homozygous triploid engrailed stock was thereby established. Engrailed intersexes are easily recognized. In them the scutellum is split characteristically, and the secondary sex-comb is commonly found. The wings of the majority are considerably shortened and rounded. In any doubtful cases—namely, extreme male types—intersexuality may be checked by an examination of the cells of the wing. In triploid females and intersexes the increased size of these cells is a clear diagnostic character.

The triploid engrailed strain and a wild type triploid stock were bred

on standard culture medium at 25°. All the cultures involved in the synthesis of the homozygous triploid engrailed stock were likewise bred under these conditions. Intersexes were collected from both triploid stocks over a period of two and one-half years, and counts were made of the number of teeth in their sex-combs in the manner previously described. The data for each intersexual class were kept separately. The mean and standard deviation together with their standard errors were calculated for each class as well as for each group as a whole.

The data obtained are recorded in tables 4 and 5. They show several important facts. First, the number of teeth in the sex-combs of intersexes

TABLE 4

Mean number of teeth in the sex-combs of wild type intersexes (N-number of sex-combs).

CLASS	N	MEAN	σ
1	116	7.76±0.09	0.95±0.06
2	110	7.93±0.10	1.08±0.07
3	40	8.13±0.10	0.86±0.10
4	132	7.80±0.09	1.02±0.06
5	88	7.66±0.10	0.96±0.07
Σ	486	7.82±0.05	1.01±0.03

is decidedly lower than in diploid males. COMBS (1937) reports mean numbers of teeth of 9.80 and 10.23 at 17° and 27°, respectively, for wild type sex-combs. The mean value for primary sex-combs of engrailed has been

TABLE 5

Mean number of teeth in the sex-combs of engrailed intersexes.

		PRIMARY		SECONDARY	
CLASS	N	MEAN	σ	MEAN	σ
1	170	8.12±0.07	0.95±0.04	2.57±0.15	2.04±0.11
2	196	8.33±0.07	0.91±0.05	1.76±0.15	2.03±0.10
3	28	8.54±0.15	0.79±0.11	1.25±0.25	1.30±0.17
4	80	8.50±0.10	0.87±0.07	1.71±0.23	2.04±0.16
5	44	8.27±0.15	0.97±0.10	3.02±0.37	2.47±0.26
Σ	518	8.29±0.04	0.96±0.03	2.10±0.09	2.10±0.06

reported above as 11.04 (25°). The means of 7.82 and 8.29 found in tables 4 and 5 obviously represent a real decrease in the size of the sex-comb. Second, in neither the wild type nor the primary engrailed sex-combs is there a significant difference between the mean number of teeth in the sex-combs of any of the five classes of intersexes. The degree of intersexuality, at least that to which the external genitalia are an index, seems to have no effect on the size of the sex-combs, since an intersex of the extreme

female type may have as many teeth in its sex-combs as one of extreme male type. Intersexes are then a uniform group in regard to their primary sex-combs.

When a comparison between the mean number of teeth in the sex-combs of wild type intersexes and the primary sex-combs of engrailed is made, it is seen that the former is significantly smaller than the latter ($d=0.37$, $\epsilon_d=0.06$). This might be accounted for in one of two ways—either the engrailed gene is directly responsible for increasing the number of teeth in the sex-comb or this increase is due to various other genetic modifiers which were brought into the wild triploid stock in the synthesis of the homozygous triploid engrailed strain. In order to test these possibilities the teeth in the sex-combs of intersexes collected from cultures used in the

TABLE 6

Mean number of teeth in the sex-combs of intersexes of intermediate genotypes.

CLASS	N	MEAN	σ
1	134	8.52±0.09	1.03±0.06
2	122	8.24±0.09	1.04±0.07
3	44	8.48±0.14	0.96±0.11
4	74	8.61±0.11	0.89±0.07
5	48	8.33±0.15	1.01±0.10
Σ	422	8.43±0.05	1.02±0.04

synthesis of this strain were counted. Such intersexes would have genetic constitutions of various intermediate conditions between those of the wild type and engrailed intersexes, and could not be distinguished phenotypically from the former. If the mean tooth number for this group should turn out to be the same as that of the wild type intersexes, it would indicate that the larger sex-comb in engrailed intersexes is due to a specific effect of the engrailed gene. If on the other hand the mean tooth number of the intermediate group should be intermediate between the other groups or as high as the engrailed intersexes, then we would conclude that the larger sex-combs in the latter have been produced by genetic modifiers brought into the triploid stock when it was outcrossed. The data in table 6 show that the latter interpretation is the more correct, since the mean number of teeth in the sex-combs of the intermediate group was actually a little higher than the mean of the engrailed intersexes, although not differing significantly from it ($d=0.14$, $\epsilon_d=0.06$). There remains the untested possibility of engrailed acting in heterozygous condition.

Further evidence for the independence of the primary sex-combs and the degree of intersexuality may be found in the data presented. If the percentage of individuals in each of the intersexual classes is computed for the

three genetically different groups of intersexes, we note that in the transition from wild type to engrailed there is an increase in male types and a corresponding decrease in female types (table 7). Class 3, which rises and then falls, is represented in each case by a rather small number of individuals. It is interesting to note that in the intermediate group there are percentages midway between wild type and engrailed, whereas these same intersexual classes were not intermediate but resembled engrailed in the condition of their sex-combs. These shifts cannot be attributed to any specific action of the engrailed gene but must be assigned to the changed genetic constitution as a whole.

The mean size of the secondary sex-combs in all five classes of engrailed intersexes was found to be less than that of diploid males reared at 25°.

TABLE 7

Percentages of individuals in intersexual classes.

CLASS	WILD TYPE	INTERMEDIATE	ENGRAILED
1	23.8	31.8	32.8
2	22.6	28.9	37.8
3	8.2	10.4	5.4
4	27.2	17.5	15.4
5	18.2	11.4	8.5

The mean for the former was 2.10 teeth, for the latter 3.57. There is a significant difference between the members of various classes—class 1 and class 5 differ from classes 2, 3, and 4. Classes 1 and 5 do not differ significantly from each other, nor do classes 2, 3, and 4. It seems strange that, if there are any differences among the classes, the extreme female type intersexes should have sex-combs of a size comparable to the most extreme male type. However, it has just been shown that genetic modifiers were responsible for the increase in number of teeth of the primary sex-combs of engrailed intersexes over that in wild type. On a more labile structure such as the secondary sex-comb it might be expected that more extreme effects of such genetic factors would be found. The combined effects of those modifying the number of teeth in the sex-comb and those which modify the degree of intersexuality may serve to produce the observed phenotype.

In the light of MRS. CURRY'S demonstration of the effect of engrailed on the genitalia, the question arises as to the accuracy of the classification of the intersexes. There is a possibility that, due to rotation of the genitalia, individuals of class 1 (extreme male type) have been erroneously placed in class 2 (abnormal male type). This, however, would not influence the conclusion that the degree of intersexuality has no effect on the size of the

primary sex-comb, nor does it affect the conclusion that there are shifts in the proportion of male and female type intersexes (table 7). Furthermore the findings regarding secondary sex-combs are not appreciably affected even if MRS. CURRY'S impression should turn out to be true that there may be an increase in the size of the secondary sex-comb in males with extremely rotated genitalia. In intersexes with abnormal genitalia this is not the case since the secondary sex-combs of class 1 were significantly larger than those of class 2 (table 5). If there were any errors in classification, they would have tended to lessen this difference.

Correlation coefficients for the number of teeth in the primary and secondary sex-combs of the engrailed intersexes were calculated and are given below. In contrast to the correlation in diploid males (table 3), these

CLASS	r
1	-0.32 ± 0.07
2	-0.46 ± 0.07
3	-0.38 ± 0.16
4	-0.49 ± 0.09
5	-0.60 ± 0.10

are all negative and, except for class 3, are all significant. There is thus a dependence of the two sex-combs on the foreleg of an intersex not found in a diploid male. Discussion of this interesting fact is reserved for a later section.

SCUTELLUM

Effect of temperature on the scutellum

Methods

Scutellar data were obtained from the same flies the study of whose sex-combs has already been discussed. In addition to 14°, 19°, 25°, and 29.5°, five other temperatures were employed—22°, 26°, 27°, 28°, and 29°. The method of rearing the flies was identical for all and has been described. For examination the adult flies were etherized and, under a binocular dissecting microscope, sorted into classes according to the extent of the cleft in the scutellum. Four classes were arbitrarily designated as follows (illustrated in fig. 5): class I, the scutellum completely divided into two separate halves;

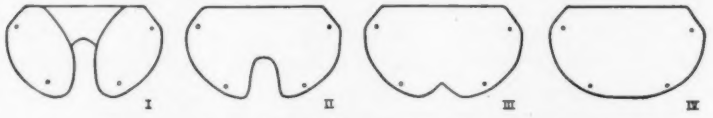

FIGURE 5.—Types of scutellar classes, I–IV. Explanation in text. Camera lucida drawings, 160×.

class II, a cleft extending beyond a line connecting the posterior scutellar bristles, but not entirely separating the two halves of the scutellum; class III, a smaller cleft, extending no farther than a line connecting the posterior scutellar bristles; class IV, no visible cleft in the scutellum but a flattening of its posterior border; completely wild type scutella with pointed posterior borders are not found. The order of these classes presumably represents the degree of fusion attained by the imaginal discs during development. There is some variability within each class, but a sharper dis-

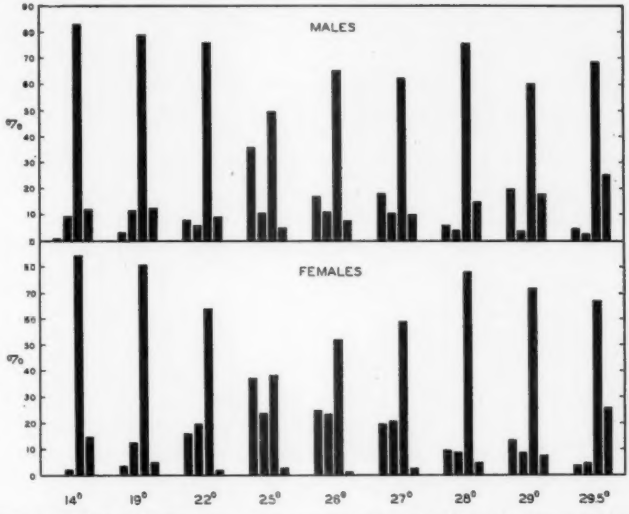

FIGURE 6.—Effect of temperature on percentage of individuals in the four scutellar classes. At each temperature classes I–IV read from left to right except for females at 14° where class I is not represented.

tinction is difficult to make, and the chance of error in placement becomes larger with a greater number of classes.

Results

The effect of temperature on the expression of the split condition of the scutellum is graphically represented in figure 6. Males and females were considered separately, since they are affected differently as determined by χ^2 calculations (table 8). In all cases, except at 29.5° and possibly at 14°, the P values are very low and thus indicate that males and females represent different populations with respect to their scutella and hence that temperature affected the cleft differently in the two sexes. In view of the sex-limited expression of the extra sex-comb it is interesting to find a sexual difference in another aspect of the expression of the engrailed gene. GOLD-

SCHMIDT (1938) has pointed out in this connection that the sex-determining mechanism of one X chromosome in the male and two in the female sets up different developmental systems in the two sexes. Therefore, not only sex-linked genes but autosomal genes as well may have a different effect in males and females. Basic developmental rates may differ or the speed of

TABLE 8

Difference of distribution of males and females into scutellar classes. ($n=3$ degrees of freedom except at 14° where $n=2$.)

TEMP.	SEX	CLASS I	II	III	IV	Σ	χ^2	P
14°	Male	1	12	108	11	132		
	Female	0	3	145	25	173	12.19	0.01
19°	Male	20	42	544	85	691		
	Female	22	89	585	34	730	27.24	0.00
22°	Male	33	25	289	34	381		
	Female	68	84	276	7	435	69.94	0.00
25°	Male	273	78	378	36	765		
	Female	278	185	295	17	775	58.82	0.00
26°	Male	34	22	133	15	204		
	Female	51	48	107	2	208	22.15	0.00
27°	Male	40	23	140	22	225		
	Female	51	54	156	6	267	20.61	0.00
28°	Male	29	16	333	64	442		
	Female	40	38	342	20	440	34.03	0.00
29°	Male	37	6	114	33	190		
	Female	30	19	163	17	229	16.90	0.00
29.5°	Male	12	7	196	72	287		
	Female	12	15	233	89	349	2.00	0.74

certain auxiliary processes may be modified in one sex more than in the other.

It must be emphasized again that the system of classification of the split scutella is an artificial one in that it leaves out of account the variability within each class. This is particularly true for classes II and III. It also divides up arbitrarily the progressive separation of the two halves without considering that this continuous series may not represent the result of a growth process whose rate is always the same. The divisions between the groups, therefore, may not coincide with the points of acceleration or of other change in the developmental reaction.

Inspection of the data in figure 6 reveals immediately that at all temperatures class III is the largest group. The distributions are different at the various temperatures except those at 26° and 27°, which are similar for both males and females. Classes I and II tend to become larger up to 25–26° and then grow smaller, while the tendency in classes III and IV is to decrease and then increase. In order to obtain a quantitative expression which would represent the average condition of the scutellum at a given temperature, the following arithmetical procedure was carried out. For each temperature the percentage of individuals in class I was multiplied by 1, that in class II by 2, and so on. The sum of these products was divided by 4 and this quotient designated as a "class average" for males and females at each temperature. These are plotted in figure 7. Treatment of the data in this way shows that there is a drop from a high class average at 14°

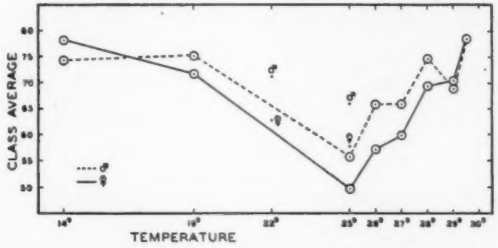

FIGURE 7.—Effect of temperature on cleft in scutellum as indicated by class averages.

and 19° to a low at 25° and then a gradual rise from 25° to 29.5°. The females give lower values than the males except at the extreme limits of the temperature range. A high class average indicates that the tendency toward an extreme cleft in the scutellum is low in the group as a whole; a low class average shows that the mean amount of splitting is somewhat higher. It should be realized that the comparatively simple picture presented in figure 7 obscures some of the enigmatical but significant fluctuations of the various classes as shown in figure 6.

When these experiments were first performed, the temperature of 22° was not employed. Later it was thought desirable to conduct a series at this temperature to determine the trend in the seemingly important interval between 19° and 25°. This was done, with a control series at 25°. The latter gave results different from those previously obtained ($\chi^2 = 94.22$, $P = 0.00$). There was a smaller percentage of both males and females in class I, and hence the class averages are higher. During the interval genetic modifiers in the purple engrailed stock had arisen by mutation or become recombined in such a way that the temperature response of the scutellum was

changed. For this reason the class averages obtained at 22° and for the controls at 25° are plotted in figure 7 but not connected to the curve drawn. These results, however, do not alter the trend of the distributions, since 22° is intermediate between 19° and 25°, and the low point of the curve still lies around 25°. The interpretation of these data will be discussed later in this paper.

Relation of body weight to scutellum

It was demonstrated earlier in this paper that body size is an important factor in the temperature effect on the sex-combs. A similar examination of the role of size in the expression of the cleft in the scutellum at a constant temperature is accordingly in order.

The flies whose body size-sex-comb relationship has already been discussed were used also for this study. Their scutellar classes were determined

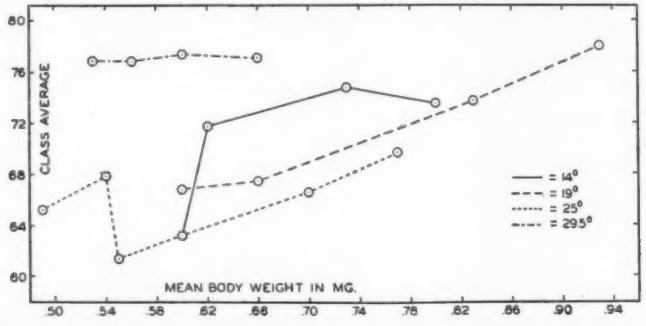

FIGURE 8.—Relation of mean body weight to extent of cleft in scutellum of males at four temperatures, as indicated by class averages for each weight group.

after weighing. Class averages for each weight group are plotted in figure 8. The significance of differences between the points at each temperature was tested by χ^2 determinations.

It was found that the rise in the curves at 14° and 19° is a significant one. Hence the scutellum tends to be less split in the larger flies at these temperatures than in the smaller individuals. At 25° the rise is not so marked, but between the low and high points there is still a significant difference and therefore still a tendency for larger flies to have more "normal" scutella. At 29.5° the nearly straight line shows that there is no effect of size on the expression of the cleft in the scutellum.

Thus the same relationship between body size and temperature effect that was found for the sex-combs holds for the scutellum. For the mutant engrailed at low temperatures there is no appreciable effect of temperature apart from its action through the body size of the fly, but at higher tem-

peratures the role of size becomes of less importance while some other phase of the temperature effect gains greater prominence in the development of the phenotype.

WINGS

Examination was made of the wings of male and female flies reared under conditions previously described at 14°, 19°, 25°, and 29°. To do this the wings were removed from the thorax and mounted directly in Canada balsam. Camera lucida drawings were then made. A study of these drawings revealed that at 14° and 19° the wings are very similar, having either large, irregular gaps in the fourth and fifth longitudinal veins or complete absence of these veins. At 25° the wings resemble those at 14° and 19° except for their smaller size. However, at 29° there is marked similarity to the wild type wing, only small gaps in the veins being occasionally found. There is thus an approach toward wild type expression in the wings with

TABLE 9

Comparison of the variability of the sex-combs and scutellum.

TEMP.	N	C	χ^2	n	P
14°	90	0.39	4.12	3	0.25
19°	324	0.14	1.53	6	0.96
25°	311	0.24	4.86	8	0.77
29.5°	283	0.30	1.39	3	0.71

increase in temperature. No quantitative study of the wing venation was attempted, since the great irregularity in the expression of the abnormalities does not lend itself readily to simple measurement.

RELATIONSHIP BETWEEN THE MULTIPLE EFFECTS OF ENGRAILED

The question now arises as to whether the changes with temperature in sex-combs, scutellum, and wings are correlated with each other. The answer to this question is a prerequisite to possible generalizations concerning the manner of action of engrailed in producing its multiple effects. Only the secondary sex-combs are used in this comparison, since the temperature response of the primaries is very slight.

In order to test for a correlation between a set of quantitative data, such as that for the sex-combs, and a set of qualitative data, as for the scutellum, the coefficient of mean square contingency (C) was calculated. The values obtained are found in table 9. There is some association between the two characters, but the coefficients are not high. It seemed desirable to determine, in this case, not how great the correlation is between the sex-combs and the scutellum, but rather how great is the lack of correlation. The χ^2 test was accordingly applied. The resulting P values are high (table 9) and

thus indicate that the observed distribution is not significantly different from a random distribution—namely, that no great degree of correlation exists between the number of teeth in the secondary sex-comb and the extent of the cleft in the scutellum at each temperature.

At 29.5° engrailed flies are most nearly wild type with respect to all three characters studied. A simple trend from extreme expression of the several effects of the mutant at low temperatures to an approach to the wild type condition at high is not evident, however. The sex-combs are most "abnormal" at 14°, the scutellum at 25°, and the wings are quite similar at 14°, 19°, and 25°.

Two mutants which resemble engrailed in their pleiotropic effects may be mentioned. The first is Polyphaen in *D. funebris*, described by H. A. TIMOFEEF-RESSOVSKY (1931), which also exhibits a lack of correlation between its various effects and a difference in the influence of temperature upon them. The second is short-wing in *D. melanogaster*, another temperature-sensitive mutant (EKER 1939). Comparison of short-wing and engrailed shows that the latter is better expressed at low temperatures, the former at high. Both show sexually dimorphic expression in some phase of the gene's effect. Whereas short-wing overlaps wild type considerably, engrailed always has 100 percent penetrance.

THE MANNER OF ACTION OF ENGRAILED

Sex-combs

The most interesting problem posed by the engrailed gene is the production of a secondary sex-comb in a specific region of the forelimb. A possible mechanism for the formation of an extra sex-comb might involve an excess either of material which can induce the development of sex-combs or of material which actually becomes incorporated in them. The disposition of this excess is such that instead of a single large sex-comb being formed a secondary sex-comb appears on the opposite side of the foreleg. There are thus specific regions in which sex-combs may arise. In this way the concept of an embryonic field may be introduced. Such a field is defined as the totality of those factors which direct development in compliance with a specific pattern.

In order to make the field concept apply to the case at hand, it may be assumed either that the sensitivity of cells to the field stimulus varies in flies of different types (as determined by genetic constitution and external environment) or that the strength of the field itself varies from type to type. In elaborating the first hypothesis it became apparent that a constant sex-comb field demands variable degrees of sensitivity of cells in different regions of the same foreleg or, in other words, two fields, one of sex-comb stimulation and one of sensitivity. The second hypothesis involves

constant sensitivity for cells of all types in all regions of the foreleg but demands various absolute strengths of the sex-comb field and also various relative strengths of its two peaks. While in reality both alternatives may contain elements of truth, only one of them, the second, will be dealt with in some detail.

This field concept is illustrated in figure 9 in which a constant threshold of sensitivity is crossed by various types of sex-comb fields. If the forelimb

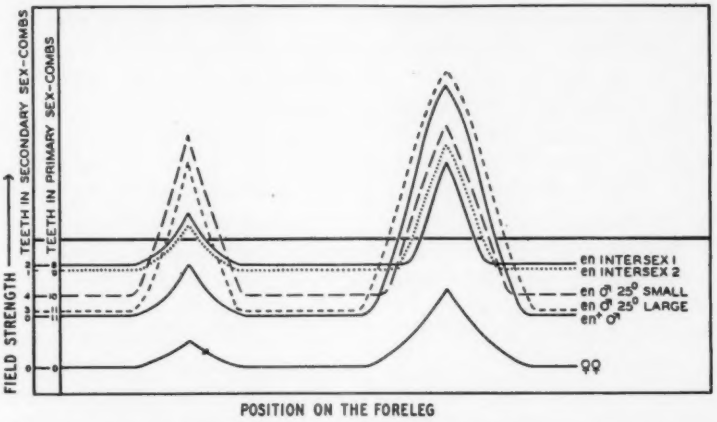

FIGURE 9.—Sex-comb fields with constant threshold of sensitivity represented by the heavy horizontal line. The numbers at the left indicate for each field the number of teeth in primary and secondary sex-combs. Further explanation in text.

of an engrailed male were split along its longitudinal axis and laid out flat, the two sex-combs would appear as structures in parallel planes. Similarly a cross section of the distal end of the first tarsal joint could be laid out, and the regions of sex-comb development would lie along two parallel lines perpendicular to the plane of the cross section. For diagrammatic purposes the field may be depicted as a curve with two somewhat conical peaks. The height of the curve at any point determines the strength of the field so that the height above the threshold of sensitivity may be used as an index of the number of teeth in the sex-comb. The area beneath the curves represents the total amount of sex-comb forming or inducing substance available.

By using a diagram such as figure 9 it is possible to give specific interpretations to the whole array of data involving different genetic constitutions—namely, femaleness, maleness, and intersexuality combined with engrailed or wild type and reared under various environmental conditions. It will be sufficient to treat only a few of these types. Wild type males

have less sex-comb forming substance than engrailed males. In the latter the added substance is distributed in such a manner that the secondary rather than the primary field is strengthened. This is also true of wild type compared to engrailed intersexes. Engrailed males differ from engrailed intersexes in that the latter have less sex-comb forming substance. While engrailed intersexes and wild type males have about the same amount of substance, its distribution is different in these two types. Females may or may not possess a sex-comb field, but if they do, as assumed in this scheme, its strength is insufficient for actual sex-comb production.

The sex-comb field for engrailed males reared at 25° varies in configuration depending on body weight. It may similarly be shown that the sex-comb field of engrailed males varies with temperature due to the amount of sex-comb forming material present. This is evident if a comparison is made of the sums of the mean number of teeth in both sex-combs at the four temperatures which are respectively 16.68, 16.22, 14.61, and 11.05 at 14°, 19°, 25°, and 29.5°. Again not only the general field strength but also the relative height of the two peaks would be affected.

It has been previously pointed out that there is no correlation between the sizes of the two sex-combs on the same foreleg in engrailed males (table 3). In intersexes, on the other hand, there is a significant negative correlation between these sex-combs, such that when one sex-comb is large the other is small. Both of these relations may be explained by assuming that the two sex-combs depend on the same material for their formation. For diploid males the total amount is great enough so that the two sex-combs may make use of it independently of each other. In intersexes the amount of material is reduced with the result that there is competition for it in the formation of the sex-combs and thus a negative correlation between their sizes.

In conclusion it may be said that whereas the above suggestions and diagram may be considered as no more than hypotheses, they may help to visualize the place of gene action in developmental processes. Definite information as to the validity of the hypothesis might be gained from observation of gynandromorphs in which a foreleg is a mosaic of male and female tissue. If a sex-comb should appear in a region composed of female tissue but surrounded by male tissue, then it might be said that a sex-comb field was present and sex-comb formation persisted in spite of the female constitution of the responding tissue. A search for such material has thus far revealed no crucial case.

Scutellum

In order to understand the complex variability of the scutellum in engrailed, an accurate knowledge of its development will be necessary. Lack-

ing such a study, only a general discussion will be attempted. We may consider two processes which are possibly concerned in the abnormal development of the scutellum—the growth of the imaginal discs and the formation of the chitinous exoskeleton. If it is assumed that these processes are dependently controlled,—that, for instance, the latter proceeded faster than the former,—the scutellum would become chitinized before its two halves were able to grow together. If growth of the imaginal discs proceeded more rapidly than chitinization, then a more complete fusion of the halves would be possible. Superimposing upon these processes (which may have different temperature coefficients as well as different rates) a variety of incubation temperatures, it is still conceivable that a fairly simple interpretation may be given for the results obtained. For instance, it may be assumed that the rate of growth of the imaginal discs increases with increasing temperature either linearly or logarithmically while the rate of chitinization increases more rapidly and reaches a maximum at 25°. This combination would produce a larger number of individuals with no cleft or only a small one at the extreme temperatures and greater splitting in a majority of those at intermediate temperatures.

The pleiotropism of engrailed

The developmental basis of pleiotropism is still largely unknown. While it may be possible to show sometimes that manifold effects are consequences of one ontogenetic mechanism ("spurious" pleiotropism, GRÜNEBERG 1938), it does not follow that those cases are "genuine" in which the reduction of apparently different effects to a common process has not been accomplished. It may be tempting to regard on the one side the effect of engrailed on both wing and scutellum as correlated ontogenetically in view of their development from the same imaginal disc and on the other side to regard the effect on the sex-comb system as independent in view of its origin from a different disc. However, at present it seems purely speculative to interpret the association data given earlier in terms of primary action of the engrailed gene.

ACKNOWLEDGMENTS

I should like to express my appreciation to DR. CURT STERN for his interest, encouragement, and many helpful suggestions throughout the course of this work. I am also indebted to DR. DONALD R. CHARLES for assistance with the statistical analyses.

SUMMARY

The principal effects of the mutant "engrailed" in *Drosophila melanogaster* are described, including a hitherto unreported character produced by this gene—an extra sex-comb on the lateral side of the first tarsal joint

of the foreleg. All of these are variable in their expression. An analysis of this variability shows that:

The mean number of teeth in the primary sex-comb is little influenced by temperature while that of the secondary sex-comb decreases with increasing temperature.

There is no correlation between the primary and secondary sex-combs on the same foreleg and low or insignificant right-left correlation between the number of teeth in the primary and secondary sex-combs.

At a constant temperature the primary sex-combs increase in size with the size of the fly in a linear fashion; the secondary sex-combs do likewise at 14°, but at 19° and 25° smaller flies have a larger mean tooth number, and the decrease in size of the sex-comb with increase in body size is not linear.

The extent of the cleft in the scutellum is influenced by temperature, with the greatest amount of separation of the two halves occurring at 25° and less splitting at the extreme temperatures.

There is less separation of the halves of the scutellum in large flies than in small ones, except at 29.5° where body size has no effect.

There is no appreciable correlation between the trend of the variability of the sex-combs and the scutellum.

Studies of sex-combs in intersexes revealed that:

The number of teeth is lowered in both wild type and engrailed.

The degree of intersexuality, as indicated by the external genitalia, has no influence on the number of teeth in the wild type sex-comb or on the primary of engrailed, but there are significant differences in mean tooth number between some of the intersexual classes in the secondary sex-combs.

There are significant negative correlations between the primary and secondary sex-combs on the same foreleg in four of the five intersexual classes.

Studies of the scutellum show that:

There is sexual dimorphism in the response to the temperature effect on the scutellum.

The influence of a body size factor in the temperature effect is greatest at low temperatures. At higher temperatures its influence is less apparent. This is true likewise for the primary sex-combs.

The concept of a developmental field is introduced as a basis for an interpretation of the effects of engrailed on the sex-combs.

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PARTHENOCARPY IN A TOMATO DEFICIENT FOR A PART OF A CHROMOSOME AND IN ITS ANEUPLOID PROGENY[1]

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INTRODUCTION

A VERY small tomato plant occurred in F_1 from diploid dwarf d_1d_1 parents, grown in the field at Riverside, California, for a study of male sterility. The mutant was very slow-growing, compared with normal sibs, but was perfectly developed and apparently healthy. It had extremely short internodes and very small leaves (fig. 1). It flowered freely and produced very small yellow (*ry*) fruit in abundance throughout the season,

FIGURE 1.—Deficient tomato plant (right) and diploid of the same family (left), in the field.

although there was, on the average, very little pollen, many anthers being completely empty. The fruit was spherical or obovoid and sometimes pointed at the stylar end. Only two of the fruits formed in the field without artificial pollination were seedy; these contained two and four seeds respectively.

After transfer to the greenhouse, nearly all the anthers of the mutant plant contained some normally matured pollen, although in flowers which had just opened about 75 percent of the pollen grains were empty; and of those grains which appeared to be normal, a great many germinated

[1] Paper no. 428, University of California Citrus Experiment Station, Riverside, California.

before anthesis. The mutant set fruit freely without artificial pollination; most of these fruits were seedless or contained one or two seeds. When artificially selfed, the fruits averaged about eight seeds each. The male-fertile diploid sibs of the mutant were very fruitful and normally seedy; the male-sterile sibs, whose pollen seldom becomes fully mature, had little or no fruit (LESLEY and LESLEY 1939).

Pollen mother cells of the mutant were studied from acetocarmine smears and from permanent smears fixed in Navashin's fluid and stained in gentian violet. Many pollen mother cells had degenerated long before anthesis, and those which underwent reduction did so at very different rates. Occasionally the shortening of the chromosomes also occurred very unevenly, since newly paired threads and fully shortened, dumb-bell-shaped pairs were found in some nuclei. These indications of internal unbalance were associated with other abnormalities in reduction. At diakinesis, there were 11 normal pairs and one unequal pair of homologues Figure 2, A to D, shows that one of the unequal homologues is about a third shorter than the other. This size difference may be seen at first metaphase. Apparently the mutant plant was a deficiency heterozygote. It has been pointed out by STADLER (1933) that in a deficient maize plant the unpaired portion of the chromosome shortens more slowly than the paired portion. It is, therefore, difficult to be sure of the exact difference between the two homologues in the deficient tomato, but it is certainly considerable.

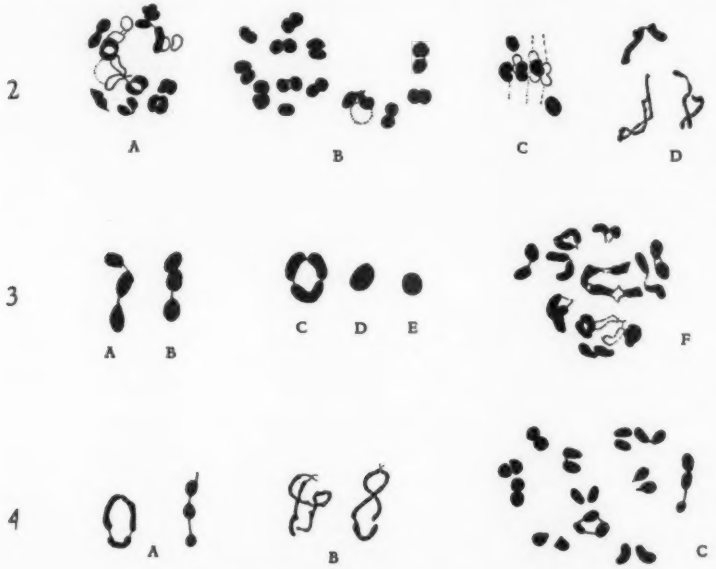
The unequal homologues were never seen to be attached at both ends at early diakinesis and were very often quite unpaired or very weakly joined at later diakinesis and first metaphase (fig. 2B, C). In the few figures seen at diplotene, the longer homologue was closely associated with the shorter throughout its length (fig. 2D). At first anaphase, the unequal homologues usually passed to opposite poles; often, before any of the other chromosomes had divided, they lay at opposite poles of the spindle. They very often lay diagonally across the plate (fig. 2C), and were sometimes in the cytoplasm at one side of the spindle. Both unequal homologues frequently passed to the same pole. Occasionally other chromosome pairs showed weakened pairing. The two chief causes of pollen abortion in the mutant plant were (1) abnormal early development of the pollen, largely dependent on environmental conditions, since in most of the smears made in the greenhouse the pollen cells were far less abnormal than in those made in the field, and (2) abnormal distribution of the members of the unequal pair of homologues.

The A or nucleolar pair of chromosomes may be heteromorphic as a result of addition of genetically inert material to the longer member, and tomato races exist which seem to differ only in the size of the A chromosomes (LESLEY and LESLEY 1935). The heteromorphic pair in the mutant plant

described above is not associated with the nucleolus; the extreme dwarfing, defective pollen, and parthenocarpy of the plant indicate that euchromatin is involved.

TRISOMIC AND TETRASOMIC DERIVATIVES OF THE DEFICIENT PLANT

The seeds obtained from selfing the mutant gave 61 diploids, six trisomics, one tetrasomic, and four runt-like plants which died before their chromosomes were examined. No plants like the original mutant occurred. The trisomic type had two long chromosomes that were nearly always closely paired and one short chromosome similar to the shorter homologue



EXPLANATION OF FIGURES 2–4

Chromosomes in pollen mother cells of tomato.

FIGURE 2.—Meiosis in the deficient plant. Unequal homologues: A, paired at diakinesis; B, unpaired and at opposite sides of the nucleus (four pairs moved to the right); C, at opposite poles at first metaphase; D, at diplotene and early diakinesis. ×3070.

FIGURE 3.—A, trivalent in a deficient trisomic; B, trivalent in a primary trisomic from a deficient trisomic selfed; C, ring trivalent from the secondary trisomic; D, typical unpaired chromosome from this secondary; E, typical unpaired chromosome from a deficient trisomic; F, diakinesis in a deficient tetrasomic plant. (A typical tetravalent is near the center of the nucleus.)

FIGURE 4.—A and B, typical ring and chain trivalents in a secondary originating from a double trisomic, triplo-BC; C, diakinesis in a hybrid between this secondary and a plant with an extra C fragment.

of the heteromorphic pair of the parent. The short chromosome was often separate, or it was more or less closely joined to the long pair (fig. 3A). If the original mutant had been the result of duplication, one would have expected that the normal diploid progeny would have two short chromosomes and that the trisomic would have two short and one long chromosome. The erratic behavior of the shorter homologue, both in the original mutant and in the trisomic, indicates that it does not undergo enough crossing over to insure association at metaphase. Evidently the longer chromosome of the unequal pair is normal and the shorter one is deficient. The appearance of the heteromorphic pair at pachytene in the deficient plant indicates that the deficiency is terminal. Terminal deficiencies have been described in *Zea mays* (McCLINTOCK 1931) but rarely occur in Drosophila (STURTEVANT and BEADLE 1939).

Since the trisomic type contained one deficient chromosome in addition to 12 normal pairs, it will be termed "deficient trisomic." The deficient trisomic plant was slightly smaller than its diploid sibs and had shorter internodes but was equally fruitful. The leaves were smaller, although not so small as those of the deficient diploid or original mutant plant, and the lamina was inclined to roll upward. Meiosis in deficient trisomic plants was about like that in the most fertile primary trisomics of the tomato. Frequent lagging and occasional omission of the deficient chromosome were observed. Rarely, all three members of the trisome were separate at diakinesis. Throughout the season an abundance of normal-looking pollen was present in anthers from pollen-fertile deficient trisomic plants.

The deficient diploid, as female parent, crossed with normal diploid gave two fruits, which together contained 47 seeds. In the F_1 grown in the field, there were 21 diploids, five deficient trisomics, and one plant almost as small as the deficient diploid and resembling it but less fruitful. This plant appeared to be a secondary trisomic since its pollen mother cells contained a ring composed of two normal-sized and one distinctly larger chromosome (fig. 3C). Since the large chromosome had a median attachment constriction, it was probably formed by duplication of the longer limb of the normal chromosome of this pair. The larger chromosome of the trivalent group was often separate at first metaphase (fig. 3D).

Only one other secondary has been found in our tomato cultures; in it, the altered chromosome was shorter than the normal chromosomes in the ring (fig. 4A, C). This secondary originated from a double trisomic (triplo-BC×diploid). Primary trisomics, diploids, a plant trisomic for a fragment of the C chromosome, and a tetraploid occurred also in the same family. This secondary showed little resemblance to its trisomic sibs. In Datura, secondaries most frequently occur in progenies of related trisomics, rarely in those of diploids. Reversed crossing over has been suggested as an ex-

planation of the occurrence of secondaries (BELLING and BLAKESLEE 1924). In a trisome at diakinesis, two of the homologous chromosomes are usually closely paired, and the third is either weakly attached to one member of the pair (fig. 3B) or entirely separate, presumably because in one member of the trisome crossing over has been greatly reduced or has failed to occur. The occurrence of a secondary in the progeny of the deficient diploid ♀ ×diploid ♂ suggests that when a secondary chromosome is formed in a diploid, the chromosome involved has already been altered in such a way as to reduce crossing over with its homologues.

Evidence has been presented by RHOADES (1940) which indicates that numerous secondaries of chromosome 5 of maize arose at meiosis as a result of the instability of the centromere of a telocentric chromosome. He is not certain whether the misbehavior of the centromere consists of failure to divide or of a transverse instead of a longitudinal division; but since UPCOTT (1937), KOLLER (1938), and DARLINGTON (1940) have observed transverse division of the centromere in lagging chromosomes, RHOADES suggests that the latter is the more likely theory. Since the deficient diploid tomato plant produced a secondary trisomic among its few progeny and only one other has been found in the tomato and since the deficient chromosome tends to behave abnormally, it seems possible that the deficient chromosome is telocentric.

The occurrence of deficient trisomics from deficient diploid×diploid is explained by the frequent passing of both members of the heteromorphic pair to the same pole.

In the two progenies from deficient trisomics selfed, there were 42 diploids, 34 deficient trisomics, five primary trisomics, three deficient tetrasomics, and one runt. One primary and two deficient trisomics produced very little fruit and proved to be male sterile. The primary type is so much like the deficient trisomic that in families segregating for leaf characters, it is usually possible to differentiate them only by cytological examination. The tetrasomics were almost as small and stunted as the deficient diploid. A deficient tetrasomic selfed gave one tetrasomic, 11 trisomics, and one diploid. The deficient chromosome is probably transmitted to about 50 percent of the progeny from selfing a deficient trisomic and is transmitted, although much less frequently, by the pollen.

In the deficient tetrasomic, meiosis was far more regular than in the deficient trisomic, but the pair of deficient chromosomes showed weakened association, as did the pair of C fragments described in an earlier paper (LESLEY and LESLEY 1929). About 40 percent of the pollen grains in freshly opened flowers were found to be empty. At the same time, about five percent of the pollen grains in freshly opened flowers of diploid derivatives of the deficient diploid were empty. Loss of unpaired deficient chro-

mosomes would result in haploid gametes and would account for the occurrence of diploid progeny from tetrasomic female × diploid male. Quadrivalents were frequent at diakinesis and first metaphase. The deficient

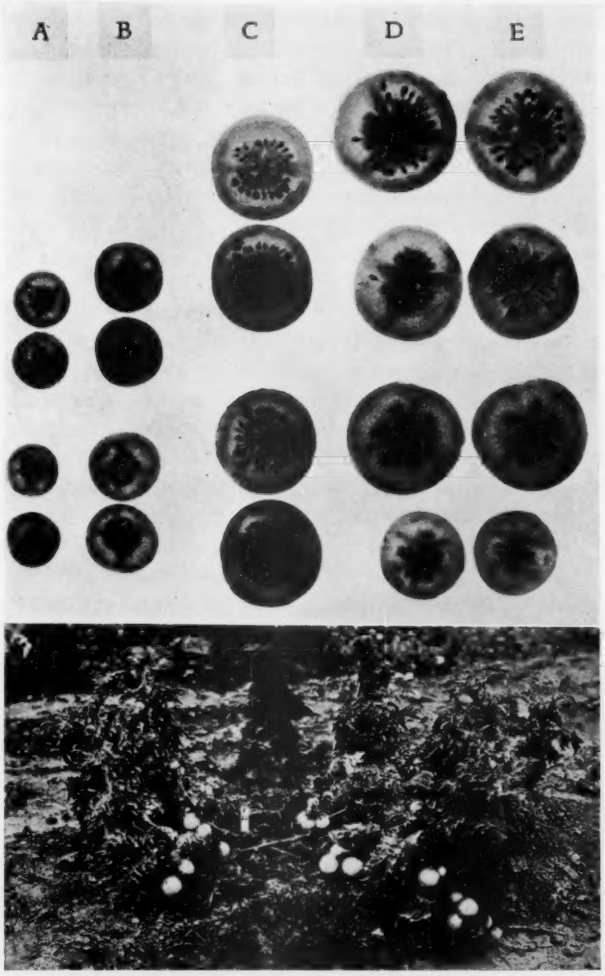


FIGURE 5 (above).—Tomatoes cut in half to show relative seediness of basal fruits (two lower rows) and apical fruits (two upper rows) of different types: vertical row A, deficient; B, deficient tetrasomic; C, diploid; D and E, deficient trisomic.

FIGURE 6 (below).—A deficient trisomic with a basal cluster of small seedless fruits (at right of label) and apical clusters of more or less seedy fruits.

chromosomes were usually attached to the two ends of a chain, as in figure 3F. Theoretically, a ring of four chromosomes might occur, but this was not found.

The very small size of the deficient diploid seems to indicate that a monosomic condition of certain genes, resulting from the deficiency, is by no means equivalent to a disomic condition in its effect on the growth of the plant. As yet, no gene has been located in the chromosome concerned in the deficiency. The primary trisomic from the deficient trisomic resembles triplo-J, which is probably a primary type, in being brachytic and unusually fruitful for a trisomic; but in a tendency to parthenocarpy it perhaps more closely resembles triplo-C and the trisomic containing a small C fragment. The deficient chromosome and the small C fragment may possibly be complementary parts of the C chromosome.

PARTHENOCARPY

Tomato trisomics vary in fruitfulness according to type and gene constitution, but when grown in the field, they are usually distinguishable from diploids by their unfruitfulness. Even when the extra chromosome present is less than normal in size, as in the deficient trisomic, an appreciable reduction in fruitfulness usually results. The deficient diploid, however, and its male-fertile deficient trisomic, deficient tetrasomic, and primary trisomic progeny were scarcely less fruitful than their diploid sibs, which were very fruitful. The unusual fruitfulness of these chromosomal mutants is apparently due to their tendency to parthenocarpy.

The fruits of the deficient diploid and of the deficient tetrasomics, both on seedlings and on plants grown from cuttings, which set without artificial pollination, were usually seedless. The secondary trisomic produced only two fruits, both seedless. In seedlings and cuttings of deficient trisomics and of primary trisomics derived from selfing the deficient diploid or from crossing it with a diploid dwarf, d_1d_1, the fruits which developed from the first or basal inflorescence of the main shoot and of the large branches originating from the lower nodes were largely parthenocarpic. The apical fruits that set on the later-developed shoots were mostly seedy (fig. 5). Some fruits were intermediate, having some seedless loculi. The seediness of basal and apical fruits of deficient trisomics and their diploid sibs is shown in table 1. As a general rule, fruits that were deficient in seed content contained more seeds in the apical half than in the basal half (fig. 5, compare D and E). Seediness of fruit tended to increase gradually from the basal to the apical regions of the plant. Diploid sibs of the trisomics and tetrasomics in most families had fewer basal fruits than the deficient trisomics, and most of these fruits were seedy. For example, in six families, the mean number of fruits on the basal inflorescence of the 97 diploid plants

was 1.7, and that of the 119 trisomic plants was 4.3; the corresponding mean proportions of basal flowers that set fruit were .23 and .37. Basal seedless fruits averaged much smaller than seedy fruits (fig. 6) but were variable in size and sometimes as large as seedy fruits.

Parthenocarpy was much more frequent in fruits that matured in summer than in those that matured in November, at Riverside. Parthenocarpic fruits occur on diploid plants, especially in the greenhouse (BOUQUET 1931). In our cultures such fruits occurred occasionally on diploid plants, but much less frequently than on the trisomics. The larger parthenocarpic fruits found on the deficient diploid and on its aneuploid derivatives were solid; the smaller fruits, which were less common, were hollow and contained no pulp. The hollow type was perhaps more frequent than the solid on diploid plants.

When unopened buds of the deficient diploid plant or of the deficient trisomic were emasculated, none set fruit even under greenhouse conditions that were very favorable to fruit setting. Application of male-sterile pollen, which mostly fails to mature and hence rarely germinates, did not cause fruit setting in these types. The parthenocarpy of the deficient diploid and of the deficient trisomic depends, therefore, on the presence of some pollen capable of germination and, in the terminology of WINKLER (1908), is stimulative. On the deficient tetrasomic, fruit set fairly readily without pollination (after emasculation); parthenocarpy in this case is vegetative. Fruits that set without pollination required about half as long again to reach maturity as seedy fruits on the same plant. They attained only about a quarter of the size of seedy fruits on the same plant and had a dull appearance. It seems probable that the presence of seedless basal fruit on deficient trisomics in midseason is due to a similarly slow development. When any of the parthenocarpic types were pollinated with male-fertile pollen, the fruits were seedy.

The smaller amount of pollen deposited naturally on the stigma seems to be favorable for the parthenocarpic development of fruit. Parthenocarpy was more common in the types having less viable pollen. This condition was confined to the basal fruits of the trisomic types but extended to all the fruits of the deficient diploid and of the deficient tetrasomics. In the deficient diploid, much less than 25 percent of the pollen was capable of germination. In the tetrasomic, although 40 percent of the grains appeared to be good, most of them contained an extra chromosome. And in the trisomic, probably more than 50 percent of the pollen contained an extra chromosome, the remainder being normal haploid. Other trisomic tomatoes, such as triplo-A and triplo-H, have only 50 percent or less of normal haploid pollen, but these types are distinctly unfruitful and non-parthenocarpic. Apparently all the parthenocarpic types have an especial

TABLE 1

Seediness of basal and apical fruits per plant in deficient trisomic and diploid tomatoes. All the basal fruits (those that set on the first or basal inflorescence) were examined, but only a part of the apical fruits (those that set on inflorescences that developed later).

PLANT PARENTAGE AND NO.	BASAL FRUITS			APICAL FRUITS		
	SEEDLESS (PARTHENOCARPIC)	PARTIALLY SEEDY	SEEDY	SEEDLESS (PARTHENOCARPIC)	PARTIALLY SEEDY	SEEDY
Deficient trisomic C376-2 selfed						
Trisomic plant no.						
2	0	0	3	0	0	10
3	11	0	0	2	2	1
4	3	1	0	0	5	0
11	10	1	11	0	13	5
14	6	2	0	0	2	8
15	13	0	0	0	2	8
17	3	2	0	0	5	11
20*	3	4	1	2	9	1
21	0	0	0	0	6	4
22*	8	0	0	5	2	0
23	2	0	4	0	0	10
24	1	0	0	0	2	10
30	4	0	0	0	0	10
32	2	0	0	3	2	3
Diploid plant no.						
1	0	0	2	0	0	14
5	0	0	3	0	1	9
7	0	0	2	0	0	10
8	0	0	0	0	0	10
9	0	0	0	0	0	10
10	0	0	0	0	0	10
12	0	0	0	0	0	10
16	0	0	0	0	0	10
18	0	0	0	0	0	11
19	0	1	2	0	1	14
25	0	0	3	0	0	10
26	0	1	0	0	0	10
27	0	0	1	0	0	10
28	0	0	1	0	0	11
29	0	0	1	0	0	10
31	0	0	6	0	0	10
33	0	0	5	0	0	10
34	0	0	2	0	0	10
Deficient tetrasomic X diploid C423						
Trisomic plant no.						
1	3	0	6	0	0	10
4	0	3	9	0	1	9

* Plant male sterile.

TABLE 1—*Continued*

PLANT PARENTAGE AND NO.	BASAL FRUITS			APICAL FRUITS		
	SEEDLESS (PARTHENOCARPIC)	PARTIALLY SEEDY	SEEDY	SEEDLESS (PARTHENOCARPIC)	PARTIALLY SEEDY	SEEDY
6	0	0	7	0	0	10
8	0	0	10	0	3	7
10	0	2	7	1	1	10
13	0	0	11	0	0	10
Diploid plant no.						
7	0	0	8	0	0	10
9	0	0	7	0	0	10
12	0	0	5	0	0	10

tendency to fruit setting. This occurs under the stimulus of a small amount of viable pollen or, in the deficient tetrasomics, even without pollination.

Trisomic plants of different genotypes varied widely in their tendency to parthenocarpy. On some trisomics even the basal fruits were seedy; on others, not only the basal but most of the apical fruits that ripened in midsummer were parthenocarpic. In a small F_1 family, C 423 (table 1), from a dwarf d_1d_1 deficient tetrasomic with basal parthenocarpy crossed with a standard D_1D_1 diploid, most of the trisomic progeny, although having plenty of basal fruits, were nonparthenocarpic. A similar condition occurred in C 422, an F_1 of a parthenocarpic primary trisomic crossed with the same standard diploid, and in a small F_2 family from C398-2, a standard D_1d_1 deficient trisomic. These three families contained the genes *R*, *Y*, and D_1 that were not present in families in which all the trisomics were partially parthenocarpic.

A primary trisomic plant, of which the basal fruits were mostly parthenocarpic, and a nonparthenocarpic primary trisome were selected from family C 422 and were crossed with the same parthenocarpic deficient trisomic. It appears from table 1 that parthenocarpy was more frequent in the progeny of the parthenocarpic primary trisomic parent.

It is not known why most of the basal fruits on certain deficient trisomic plants were parthenocarpic. The fruiting of White San Pedro fig, described by CONDIT (1938), is somewhat analogous, the first crop being parthenocarpic and the second nonparthenocarpic. Evidently in the tomato the fruits of the deficient diploid and of its aneuploid progeny are seedy if conditions are favorable for fertilization. Since growth-promoting substances cause tomatoes to set parthenocarpically (GUSTAFSON 1936), it is possible that the deficient diploid and its trisomic and tetrasomic derivatives have a higher auxin content than the diploids. Unlike the partheno-

carpic fruits which GUSTAFSON produced with auxins, the seedless fruits in our cultures were, as a rule, smaller than the seedy fruits. Presumably these seedless fruits did not receive so much growth-promoting substance as the seedy fruits.

Seedlessness might result from the effect of high temperature on pollen (SMITH and COCHRAN 1935). HAWTHORN (1937) reports that in Texas certain derivatives of Large Cherry × Bonny Best varieties of tomato produced seedy fruits early in the season, then seedless fruits during a period of very high temperature, and seedy fruits again late in the season. LOEHWING[2] finds that, in general, high temperature and long day favor parthenocarpy and that in most tomato varieties, day lengths in excess of 12 hours are necessary to the parthenocarpic development of fruits. The

TABLE 2

Parthenocarpy in progenies of a parthenocarpic primary trisomic tomato and of a nonparthenocarpic primary trisomic sib, both crossed with the same parthenocarpic deficient trisomic. P = parthenocarpic; SP = slightly parthenocarpic; NP = nonparthenocarpic.

	TRISOMIC PROGENY			DIPLOID PROGENY		
	P	SP	NP	P	SP	NP
Parthenocarpic primary trisomic × nonparthenocarpic deficient trisomic, 39010.1	12	10	1	0	5	13
Nonparthenocarpic primary trisomic × nonparthenocarpic deficient trisomic, 39010.1	6	9	6	0	0	20

average mean daily temperature at Riverside in June 1939 was 71.1°F; this was 5.8° lower than the average in July, but the setting of the parthenocarpic fruit of the deficient trisomic probably occurred during June, or mainly then.

The parthenocarpic tendency of the deficient diploid plant and its aneuploid progeny indicates that the same chromosome is involved in all. Apparently in the whole chromosome the gene balance is favorable to parthenocarpy. This favorable effect is increased by the presence of an extra whole chromosome of this type, as in the simple trisomic. In the deficient chromosome, the gene balance is even more favorable to parthenocarpy, owing to the loss of genes unfavorable to it. On this hypothesis, the balance of genes in the deficient diploid, the deficient trisomic, the deficient tetrasomic, and in the primary trisomic is more favorable to parthenocarpy than in the diploid.

[2] PROFESSOR W. F. LOEHWING, UNIVERSITY OF IOWA. Unpublished data.

SUMMARY

A heterozygous deficient tomato plant originated in F_1 from diploid dwarf parents, probably from the egg cell of a deficient female gametophyte and a normal male gamete. The deficient diploid plant was very small but set seedless fruit abundantly without artificial pollination. The deficiency is equivalent to about one-third of the length of the chromosome and appears to be terminal. At diplotene the unequal homologues were closely associated, and at diakinesis they were attached at one end or were separate. The pollen, especially under field conditions, was scanty and mostly abnormal.

The aneuploid derivatives of the deficient plant included a primary trisomic, a deficient trisomic, a deficient tetrasomic, and a secondary trisomic. These, like the deficient, were relatively fruitful and had a tendency to parthenocarpy. No deficient diploids were identified in progenies from the deficient diploid plant.

Either a deficiency of part of a chromosome or the addition of the deficient chromosome or of the whole chromosome to the normal complement causes a tendency to parthenocarpy.

In deficient trisomic and primary trisomic plants, the basal fruits tend to be parthenocarpic. The fruits that set on inflorescences that develop later are less likely to be parthenocarpic, and apical fruits are generally seedy. Diploid plants sometimes have a few basal parthenocarpic fruits but can usually be distinguished from trisomics by the scarcity of basal fruit and a greater tendency to seediness.

Parthenocarpy was stimulative except on the deficient tetrasomic, which sets fruit without pollination. These fruits, like the parthenocarpic fruits on the deficient trisomic, were, on the average, much smaller than the seedy fruits and required about half as long again to reach maturity. When artificially pollinated, all types of plants as a rule produced seedy fruits.

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